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EFFECTS OF MICROCLIMATIC CHANGES ON OOGENESIS OF *DROSOPHILA MIMICA*

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PREFACE

The following account reports investigations made during 1967 and 1968, three years prior to the initiation of the intensive ecological studies of the Island Ecosystems IRP of the U.S. IBP. They were carried out in exactly the same areas along the Mauna Loa Strip Road in Hawaii Volcanoes National Park where some of these later studies were made. They relate particularly to the temporal relationships of organisms in the ecosystem, the annual climatic cycle and water stress problems. These same problems have received much attention from later workers; indeed the author himself returned to these sites in order to obtain validating and supplementary data in July and August of 1972. Accordingly, inclusion of this material in the present Technical Report series is especially appropriate and will provide an opportunity for relevant comparisons. The data will be especially useful in the preparation of certain of the syntheses which are planned as part of the Island Ecosystems IRP.

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ABSTRACT

The reproductive mode of Drosophila mimica, a species endemic to the island of Hawaii, was determined by analyzing the ovarian development of three natural populations during a nine month period. Qualitatively the developmental profile of the ovaries remained the same in all the populations and for the entire collecting period. Each developmental stage was represented only in a fraction of the ovarioles, and mature eggs were usually found in half of the ovarioles. Quantitative differences were found between populations and between collections, and these differences were correlated with the environmental conditions.

The relative humidity was found to be the most important factor in regulating ovarian development by interrupting the growth of oocytes at the stage of RNA-yolk synthesis. A seven day period of constant low relative humidity causes the degeneration of grown oocytes, while a constant high humidity for the same period of time reinitiates normal development. This mechanism serves as a device to assure the presence of not more than one mature egg per ovariole, and thus prevents the overpopulation of the natural breeding substrates after environmental stresses. The adaptive significance and the theoretical implications of such behavior were discussed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

A fundamental task in evolutionary biology is to determine the mechanisms by which different modes of reproduction have evolved. Studies on clutch size in birds and lizards (Lack, 1954; Cody, 1966; Tinkle, 1969), egg production in fish (Williams, 1966) and the diverse ovarian development in Drosophila species endemic to Hawaii (Kambysellis and Heed, 1971), illustrate the variety of strategies employed to ensure survival of progeny in diverse environments. However, the extent to which these species are adapted to their environments so as to ensure egg production only under favorable conditions for the offspring has not been determined in any of the cases.

The Hawaiian Drosophila species living in relatively constant environments appear to be the most promising organisms for such a study. In the present report, one such species, Drosophila mimica, will be examined. I will determine its reproductive mode, indicate the reproductive stability of isolated populations of the species over long periods of time, and analyze the reproductive responses of the population to environmental changes. By correlating the ovarian developmental conditions of the species in their natural habitat with the existing weather conditions (cf. data accumulated by Dr. G.A. Smathers (1968)), I will show that the reproductive mode is fixed for the species. Further, I will show that the isolated populations are capable of responding to environmental fluctuations by regulating various intermediate steps of the reproductive process, to ensure egg production only during periods favorable for oviposition.

MATERIAL AND METHODS

1. Collecting localities

It was of the utmost importance in this study to have appropriate collecting localities, i.e., small geographically isolated areas which support well-established populations of related Drosophila species and provide diversity in their ecological habitats and weather conditions. The island of Hawaii, the youngest in the Hawaiian chain, was selected because recent volcanic activity has isolated several small areas of native vegetation forming the so-called "kipukas". These islands of dense tropical forest are still inhabited by their original Drosophila populations which have remained isolated from their neighbor populations by extensive areas of barren lava (Carson et al., 1970). We chose two such areas, Kipuka Puaulu and Kipuka Ki in Hawaii Volcanoes National Park, located on the slopes of the Mauna Loa Volcano at 4000' elevation (Figure 1). Mueller-Dombois and Lamoureux estimated these kipukas to have been established less than 2,000 years ago. They classified the vegetation as a closed to semi-open forest with soapberry (Sapindus saponaria) and ohia trees (Metrosideros collina) forming the overstory of the forest, pilo (Coprosma rhynchocarpa) and mamake (Pipturus albidus) the mid-layer, and the fern palapalai (Microlepia setosa) the ground layer. Kipuka Ki is rather uniform in tree density while Kipuka Puaulu provides areas with denser vegetation, often enriched with Pisonia and devoid of ferns.

In Kipuka Puaulu (KP) we selected one Pisonia containing area of approximately 2,000 square feet and designated it as collecting site number one (KPI). A small (1,000 square feet) less dense forest area, about 500 yards away and devoid of Pisonia and ferns was used as collecting site number two (KPII). Our third collecting site was chosen in Kipuka Ki (KK)

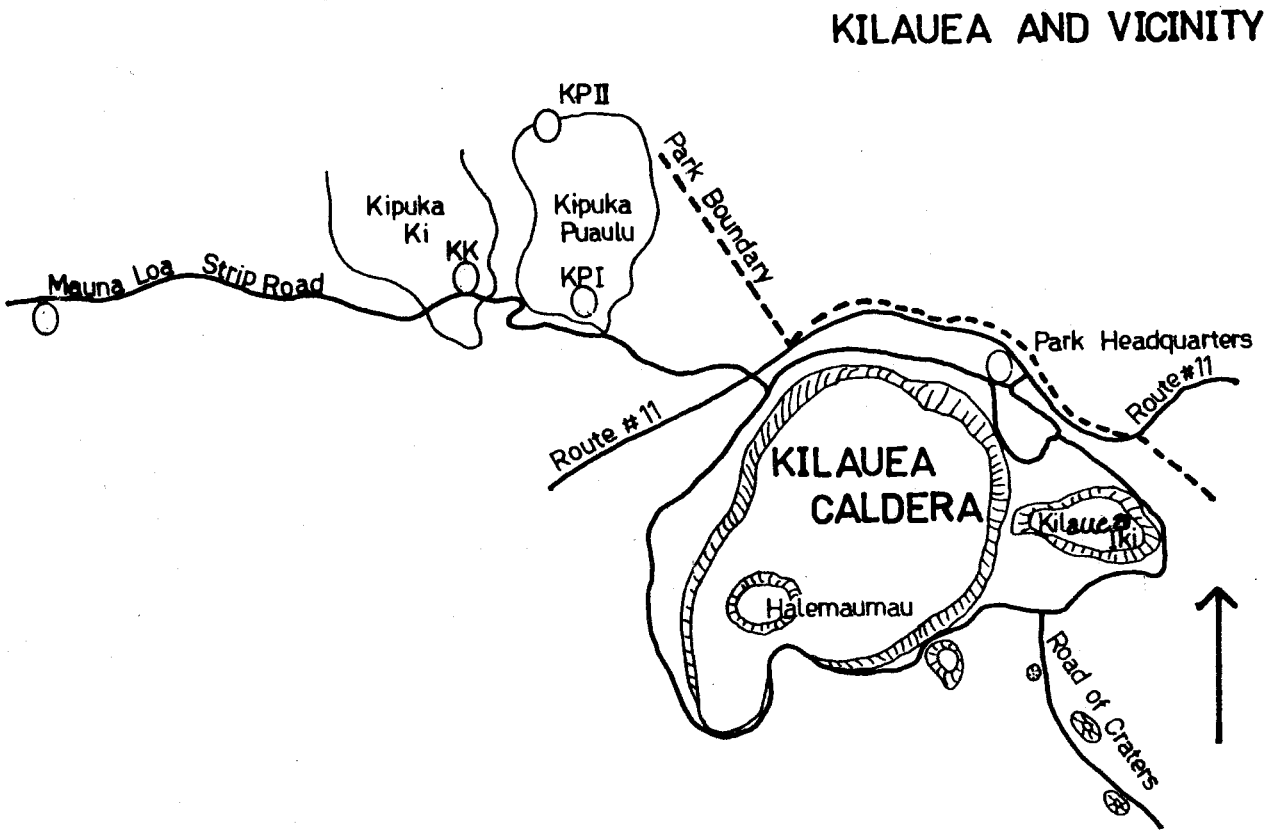

FIG. 1. Collecting localities and adjacent area.

and had a vegetation similar to KPII and an area of 2,000 square feet (Figure 1).

The kipukas in general and more particularly our collecting areas are inhabited by dense populations of endemic Drosophila species, some of which are found only in these kipukas. The most abundant species are D. mimica, D. engyochracea, and D. imparisetae. However, in the area where Pisonia trees are numerous (KPI), D. kambysellisi is the predominant species.

2. Weather Conditions

a. Temperature and relative humidity

The major meterological station was located in KPI. A Bendix Hygrothermograph (Model 594) was placed at ground level, beneath the ohia Sapindus canopy. The instrument was periodically standardized with a Bendix Psychrometer (Model 566) and its chart was changed weekly by Dr. G.A. Smathers.

Periodic temperature measurements were made along a vertical gradient of air and soil in the understory of both kipukas at the points +250 cm, +150 cm, ±50 cm, 0 (ground contact), -0.5 cm, -2.5 cm, -5.0 cm. Temperatures of Sapindus fruits (the breeding substrates for D. mimica), Sapindus leaves and tree trunks near ground (the feeding sites of the adults) were determined. All measurements were made with a Yellow Springs Telethermometer (Model 43TC), using appropriate probes for air, ground surface and soil temperatures. At the same time that air and soil temperature were measured, the relative humidity was determined with a Bendix Psychrometer (Model 566) at 250, 150, 50 and 10 cm above ground level.

b. Rainfall

Rainfall gauges (Tru-check type 510) were placed outside the canopies of each kipuka. One gauge was placed near the hygrothermograph under the

canopy at the site KPI. This gauge recorded the "through-fall" amount of rainfall. All gauges were read weekly.

c. pH measurements

A volcanic eruption of the adjacent crater Halemaumau (2.5 miles to the southeast of Kipuka Puaulu, Figure 1) during the progress of this project (Nov. 5, 1967) continued to spew acid fumes into the atmosphere. As a result rainfall on the summit and southwestern flank of Kilauea volcano was decidedly acidic and several species of native and introduced plants were killed back to ground level. Because this unusual environmental circumstance could have some effects on the Drosophila populations it was decided to keep a record of the pH of the rainfall just outside Kipuka Puaulu.

d. Potential evaporation

The rate of potential evaporation on the forest floor was determined once every week using standardized Livingston porous-sphere (white) atmometers.

3. Collecting techniques

Regular monthly collecting visits were made the 1st and 2nd day of each month between November 1967 and July 1968. Two additional collections were made in July and August 1972. Immediately after collection, the flies were stored in shell vials with food consisting of agar-Karo-syrup-distilled water solution (Spieth 1966) for transportation to the local laboratory. After species identification, (usually one to two hours after collection) the thorax length of each female fly was measured in lateral view under a dissecting microscope at a magnification of 30x. The ovaries were dissected in Waddington's Ringer solution, then fixed and stored for transportation to the laboratory in formalin-absolute alcohol-acetic acid-

distilled water (6:16:1:30 v/v). At a convenient time the ovaries were stained according to the method of Whiting (1950) and the egg chambers were identified according to King's terminology (King et al., 1956). On some occasions the females were kept singly in shell vials with laboratory medium (Wheeler and Clayton, 1965) and F_1 progeny were used to determine the growth curve of oogenesis.

4. Pattern of oviposition

A sample of 40 wild collected females was used to determine the pattern of oviposition. Each of the females was kept with two males in a separate shell vial with laboratory medium into which a saturated piece of tissue paper was inserted. Every 24 hours the vials and the paper were examined, and the number of oviposted eggs was recorded. The females were placed in fresh shell vials every fourth day. To avoid any artefacts due to the transfer onto fresh food, the population was subdivided into four groups of ten flies and only one group was placed on fresh medium each day.

RESULTS

1. Analysis of oogenesis in natural populations

The ovaries of more than 1300 D. mimica were analyzed. The monthly collections were analyzed separately for each collecting site. The collection of December 1967 at Kipuka Puaulu (KPI) will be used as an example to present the method applied for the analysis.

Each ovary consists of a cluster of parallel egg tubes, the ovarioles, (Figure 2) held together by a peritoneal sheath. The number of ovarioles varies between species and also between individuals. Each ovariole consists of a moniliform series of egg chambers at various stages of development with the anterior one being the youngest chamber and the posterior the most mature (Figure 2).

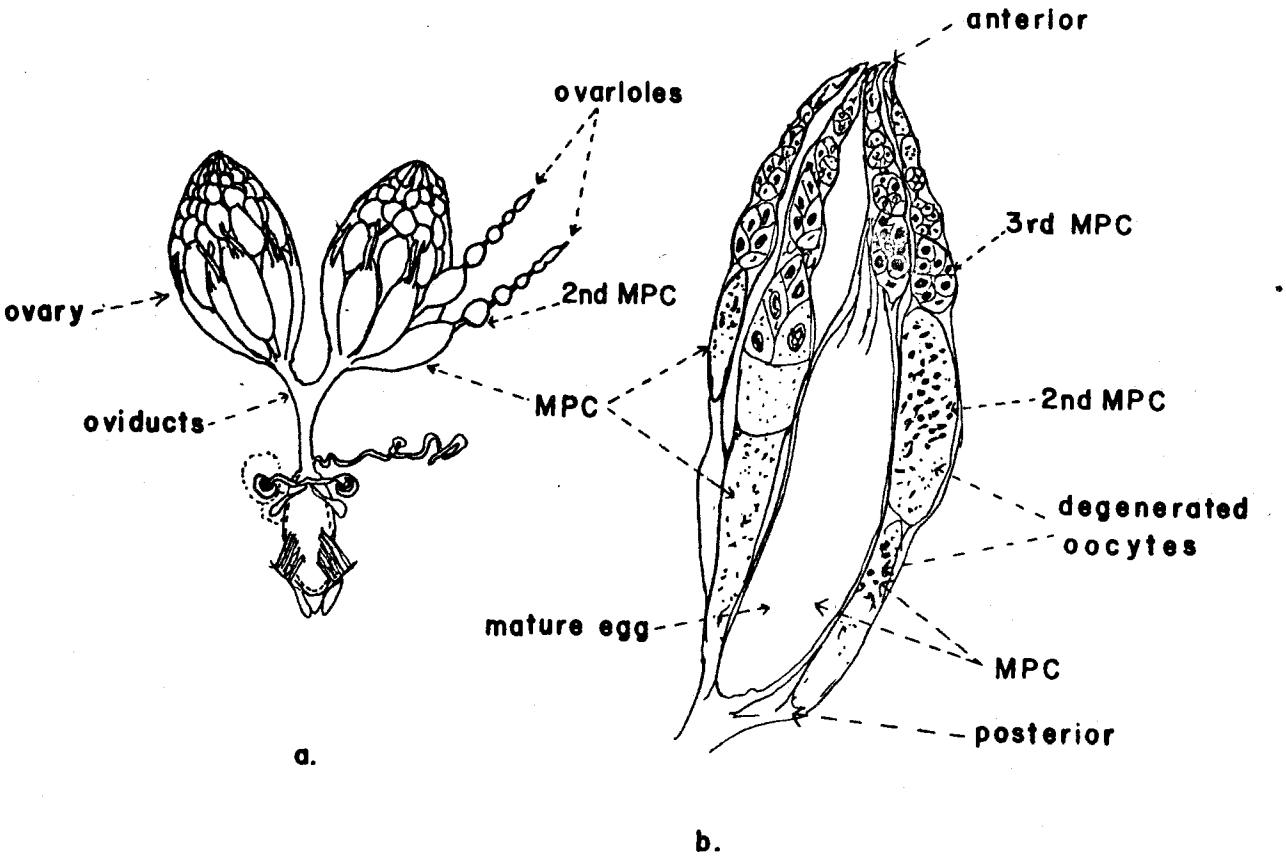

FIG. 2. Ovaries of Drosophila mimica.

a. Morphology of mature ovaries.

b. Ovarioles showing some of the developmental stages and degenerated egg chambers.

In each collection the number of ovarioles per fly was determined and the developmental condition of the egg chambers in each ovariole was recorded based on a 14 stage process with stage 14 representing the mature eggs (King et al., 1956). Only flies containing at least one mature egg were included in the final analysis and these represent the "mature fraction" of the population. The maturation of the first oocytes under laboratory conditions requires a period of 8 to 10 days (see page 23) and therefore it is safe to say that only females older than ten days were used in the final analysis. The immature flies represent approximately 22 percent of the population, and this fraction varies significantly between collections and localities (Table 1).

The number of egg chambers per ovariole in mature flies varied from four to seven with a mean of five. As a routine, for each ovariole, the four egg chambers closest to the oviduct were analyzed, and in the following, these chambers will be designated as the "Total Egg Chambers" (TEC). For each fly the relative frequencies of each developmental stage of the TEC were computed by dividing the number of egg chambers at each developmental stage by the total number of ovarioles analyzed. This analysis could give values higher that 100% in the cases in which a particular developmental stage was represented more than once in some or all the ovarioles. Then, for the entire collection, the mean relative frequencies of each developmental stage was computed, and this expresses the reproductive ability of the population at the time of dissection. The graphic presentation was designated as the "profile of ovarian development" and Figure 3a shows such a profile.

It is apparent that some of the developmental stages (S5, 7, 9) were more frequent than others (S10-13) and that each developmental stage was

Table 1. Number of D. mimica females collected during November 1967-July 1968 and July-August 1972, and frequencies of immature population for each collection.

Month of collection	Collecting Locality: Kipuka Puaulu I Number of flies collected	Kipuka Puaulu I Immature population (%)	Kipuka Puaulu II Number of flies collected	Kipuka Puaulu II Immature population (%)	Kipuka Ki Number of flies collected	Kipuka Ki Immature population (%)	Total collections Number of flies collected	Total collections Immature population (%)
Nov. 1967	42	4.8	20	0	52	7.7	114	5.3
Dec. 1967	16	12.5	48	45.8	24	16.7	88	31.8
Jan. 1968	30	13.3	58	24.1	74	10.8	162	16.0
Feb. 1968	36	5.6	46	13.0	28	28.6	110	14.5
March 1968	36	22.2	50	16.0	54	7.4	140	14.3
April 1968	32	31.2	34	5.9	56	17.9	122	18.0
May 1968	32	12.5	56	39.3	46	4.3	134	20.9
June 1968	28	50.0	56	53.6	44	45.4	128	50.0
July 1968	24	41.7	44	22.7	22	9.1	90	24.4
July 1972	37	29.7	40	35.0	42	19.0	119	27.7
Aug. 1972	77	13.0					77	13.0
Total	390	19.7	452	28.3	442	15.8	1284	21.4

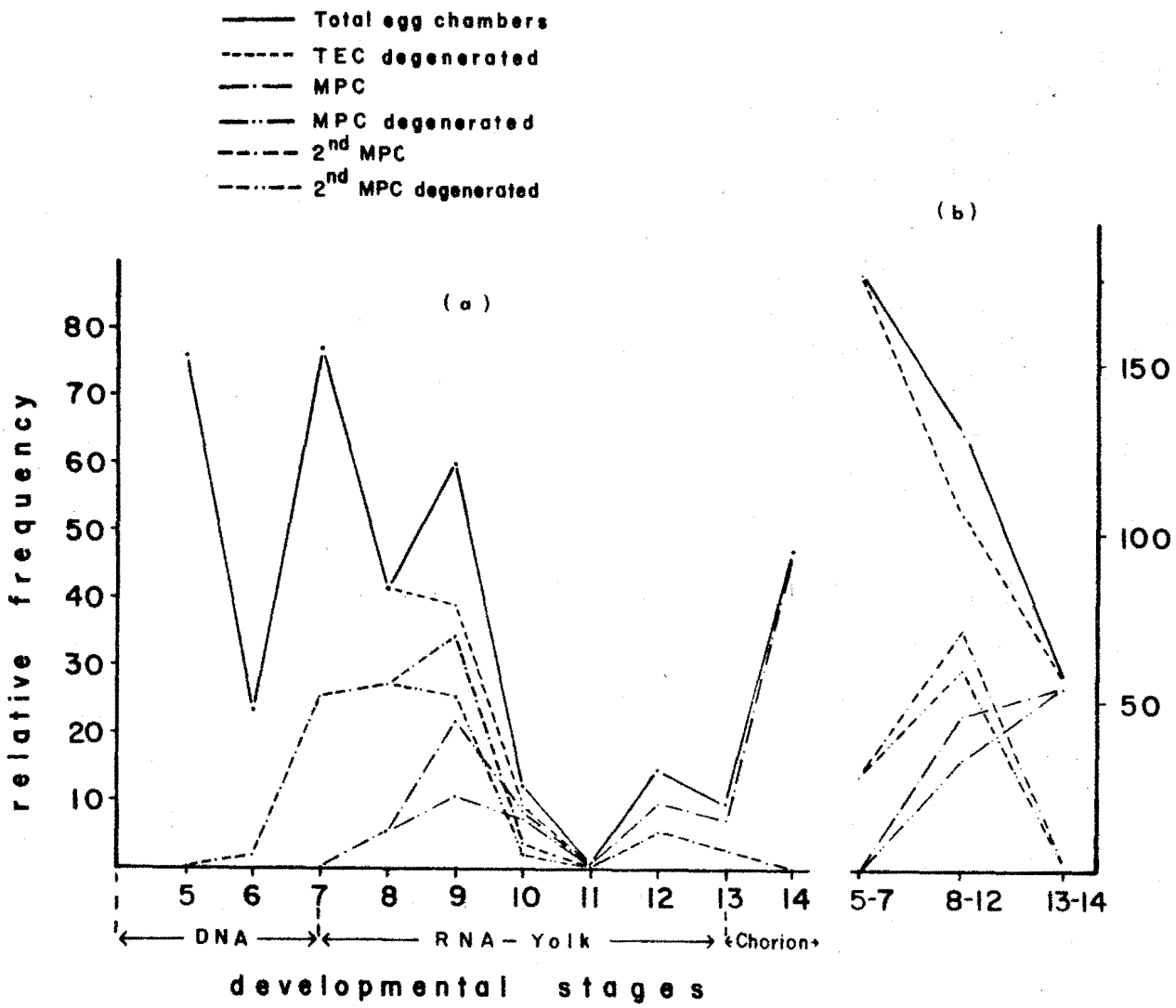

FIG. 3. The profile of ovarian development of D. mimica in Kipuka Puaulu I for December 1967. a) analytical profile based on 14 developmental stages. b) summary profile based on 3 developmental groups (for details see text).

represented only in a fraction of the ovarioles. Mature eggs (S14) were present in less than 50% of the ovarioles (47.1%), whereas younger stages (S7, S9) were always more frequent (78% and 60% respectively). Invariably, a considerable number (up to 25%) of growing egg chambers (S9-S10, Figure 2b) had nurse cells with pycnotic nuclei and/or abnormalities in yolk formation which lead to degeneration and eventually resorption of the chamber.

The profile of the development of the TEC, although it provides information on the reproductive ability of the population, does not reveal the realization or reproductive efficiency of the flies at the time of dissection. This is because it does not discriminate between egg chambers at different positions on the ovarioles and, therefore, does not indicate the realization of oviposition. To overcome these deficiencies, a similar analysis to that of the TEC was carried out for the most posterior chambers (MPC), second most posterior chambers (2nd MPC), 3rd MPC and 4th MPC (Figure 2), and their developmental profiles for the entire population were computed.

The profile of development of the MPC (Figure 3a) clearly demonstrates that all the mature eggs (S14) were localized in the MPC and, furthermore, in only less than 50 percent of the ovarioles (47.1%). This means that the ovipositing ability of the flies was restricted, and the maximum number of eggs that could be laid at a time was about half the number of their ovarioles. Of the remaining MPC, 13% were degenerated and the other 60% were distributed among young developmental stages primarily at the stage of yolk formation. The 2nd MPC (Figure 3a) were primarily young, yolk synthesizing chambers (S8-10), with a substantial number degenerated (11%).

The 3rd and 4th MPC (not shown in Figure 3) were almost exclusively at stages 7 and younger. These findings demonstrate that only a fraction of the ovarioles successfully mature their egg chambers at any one time, and that the ovarioles containing mature eggs in each ovary were alternated between successive ovipositions.

2. Annual profile of ovarian development

The observations from the December collection appeared to be qualitatively comparable to all other collections (Table 2), and the basic profile of ovarian development (Figure 3a) remained in general the same. The younger stages (S5, 7, 8, 9) were the most frequent, while the mature eggs (S14) were restricted only to a fraction of the ovarioles, and as a rule to the MPC. From the several hundreds of flies analyzed, only a few specimens with mature eggs in each ovariole was found and in one extreme case (July 1972) one individual had two eggs in some ovarioles. This remarkable observation demonstrates that although this species possesses the potential to accumulate more than one mature egg per ovariole (a behavior common to many Hawaiian Drosophila, Kambysellis and Heed, 1971), the realization is a very rare event and is accomplished only under special environmental conditions. The existence of this potential was also suggested by the observation that a small percentage of ovarioles (0.3-5.3%) always contained a second egg chamber at the final stage of egg maturation (S13, Table 2, 2nd MPC). These observations strongly suggest that the reproductive mode in D. mimica represents a transitional step between the single-egg ovariole of the leaf breeders and the multi-egg ovariole of the bark breeders (Kambysellis and Heed, 1971).

Comparable results were obtained by analyzing collections from Kipuka Puaulu II (Table 3) and Kipuka Ki (Table 4). Although qualitatively the

Table 2. Profile of the developmental condition of the ovaries of D. mimica collected in Kipuka Puaulu I during Nov. 1967 - July 1968 and July - August 1972

Month of collection	Number of flies analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage* (relative frequencies in %)										% of degenerated egg chambers (Total)
				S5*	S6	S7	S8	S9	S10	S11	S12	S13	S14	
				Total Egg Chambers (TEC)										
November	42	1090	4360	54.7	45.3	85.7(0.8)**	66.4(22.6)	19.3(15.2)	9.4(1.5)	1.7	7.0	22.8	40.0	40.1
December	28	620	2480	76.1	23.2	77.4	41.3	60.0(21.3)	12.3(3.2)	0.6	14.8	9.7	47.1	24.5
January	26	628	2512	59.2	37.9	60.2(0.3)	52.2(3.8)	38.9(12.1)	25.2(3.8)	9.9(0.3)	24.5	12.1	30.3	20.3
February	34	780	3120	96.9	2.6	98.5	66.7(2.1)	47.9(3.8)	25.6(3.3)	7.4	15.6	30.5	34.9	9.2
March	28	570	2280	63.2	36.8	62.8	45.6(2.5)	52.3(15.8)	18.6(0.7)	11.2	22.8	25.3	42.1	19.0
April	22	456	1824	98.2	2.2	97.4	46.1(2.6)	51.8(27.2)	10.5(0.4)	15.4	8.3	17.5	32.9	30.2
May	28	580	2320	99.0	1.0	99.0	45.9(5.9)	54.1(21.0)	6.9	9.3	17.9	17.9	24.5	26.9
June	14	340	1460	98.2	1.8	98.2(2.9)	67.6(28.2)	27.1(18.8)	2.9(1.8)	1.8	15.9	5.9	36.5	52.3
July	14	342	1368	98.2	1.2	98.2	49.7(14.0)	50.9(40.9)	1.2(0.6)	0	1.8(0.6)	11.1	61.4	55.5
July	52	1186	4744	68.6	31.0	72.2	65.4(16.9)	38.3(25.3)	0.8(0.3)	1.0	6.4	11.5	35.7	42.5
August	67	1623	5569	63.4	42.6	63.7(0.4)	60.4(14.9)	38.6(28.0)	3.9(0.1)	3.7	21.1	11.8	42.0	43.4
				Most Posterior Egg Chambers (MPC)										
November	42	1090	1090	0.6	0.6	0.9(0.2)	12.3(6.2)	9.5(7.7)	7.5(1.1)	1.5	6.2	20.9	40.0	15.2
December	28	620	620				5.2	21.9(11.6)	8.4(1.3)	0.6	9.7	7.1	47.1	12.9

* Identification according to King's terminology (King et al., 1956).

** Numbers in parenthesis represents relative frequencies of degenerated egg chambers.

Table 2. (Cont.)

Month of collection	Number of flies analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage (relative frequencies in %)										% of degenerated egg chambers (Total)
				S5*	S6	S7	S8	S9	S10	S11	S12	S13	S14	
				Most Posterior Egg Chambers (MPC)										
January	26	628	628				4.5	8.9(1.3)	14.0(2.5)	7.0(0.3)	23.9	11.5(0.3)	30.3	4.4
February	34	780	780				1.0	7.2	10.3(0.5)	4.6	14.1	27.9	34.9	0.5
March	28	570	570				0.4	4.2(3.9)	5.6(0.7)	6.7	18.6	22.5	42.1	4.6
April	22	456	456				4.8(0.4)	17.1(11.8)	6.1	13.6	7.9	17.5	32.9	12.2
May	28	580	580				8.6(5.2)	18.6(6.6)	5.2	7.9	16.6	17.9	24.5	11.8
June	14	340	340				18.2(8.2)	21.2(13.5)	2.9(1.8)	1.8	15.3(0.6)	2.4	36.5	24.1
July	14	342	342				6.4(3.5)	24.0(21.1)	0.6(0.6)	0	1.8	5.8	61.4	25.2
July	52	1186	1186			1.0	24.3(8.6)	20.1(15.2)	0.7(0.3)	0.5	5.9	11.8	35.8	24.1
August	67	1623	1623		0.4	0.7(0.3)	4.1(1.5)	16.3(13.5)	2.8(0.1)	3.0	19.8(0.1)	11.0	42.0	15.4
				2nd Most Posterior Chambers (2nd MPC)										
November	42	1090	1090		2.6	32.3(0.4)	49.5(15.2)	9.7(7.5)	1.8(0.2)	0.2	0.7	1.8		23.3
December	28	620	620		1.9	25.2	27.1	34.2(9.0)	3.9(1.9)	0.0	5.2	2.6		10.9
January	26	628	628	1.0	3.2	18.2	33.8(3.5)	29.0(10.8)	11.1(1.3)	2.9	0.6	0.3		15.6
February	34	780	780			7.2	33.6(2.1)	37.4(3.3)	15.1(2.8)	2.8	1.5	2.3		8.2
March	28	570	570			6.7	24.9(2.1)	43.9(11.9)	13.0	4.6	4.2	2.8		14.0
April	22	456	456			24.1	35.1(2.2)	34.6(17.1)	4.4(0.4)	1.8	0.0	0.0		19.7
May	28	580	580	0.7	0.0	28.3	31.7(0.7)	35.2(12.1)	1.7	1.0	1.4	0.0		12.8
June	14	340	340	1.8	0.0	43.5(1.8)	44.7(15.9)	5.9(5.3)	0.0	0.0	0.6	3.5		23.0
July	14	342	342			29.8	39.8(9.4)	24.6(14.0)	0.6	0.0	0.0	5.3		23.4
July	52	1186	1186	0.7	12.1	33.7	36.4(7.3)	13.5(9.1)	0.2	0.2	0.4	1.5		16.4
August	67	1623	1623	0.4	2.8	18.2(0.1)	52.3(13.0)	22.7(15.1)	1.2(0.1)	0.7	1.1	0.0		28.3

Table 3. Profile of the developmental condition of the ovaries of *D. mimica* collected in Kipuka Puaulu II during Nov. 1967-July 1968 and July 1972.

Month of collection	Number of flies analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage* (relative frequencies in %)										% of degenerated egg chamber (Total)
				S5*	S6	S7	S8	S9	S10	S11	S12	S13	S14	
				Total Egg Chambers (TEC)										
November	20	502	2008	59.4	42.2	82.5	72.1(35.9)**	8.0(5.6)	8.4(1.2)	3.6	10.4	17.5	37.5	42.7
December	26	678	2712	70.8	28.9	73.2	42.2	46.9(16.2)	10.9(2.7)	2.7	15.9	8.8	14.2	18.9
January	44	1102	4408	99.8	0.2	99.8	63.5(2.0)	48.6(25.6)	17.8(4.0)	9.8	16.7	15.2	43.0	31.6
February	40	992	3968	69.0	31.3	72.2	58.1(0.8)	40.3(2.6)	24.4(0.6)	8.1	13.9	18.1	29.8	4.0
March	42	940	3760	66.8	33.2	67.0	48.3(1.5)	48.1(18.5)	24.7(0.4)	14.0	16.8	24.3	32.8	20.4
April	32	738	2972	96.5	3.5	96.2(1.4)	43.6(3.2)	48.0(17.6)	12.2(1.1)	10.8	12.5	20.9	29.8	23.3
May	34	798	3192	95.7	4.0	96.2	51.1(7.0)	48.1(22.8)	6.3	6.3	17.8	20.1	32.1	29.8
June	26	614	2456	98.0	1.3	99.0	70.4(39.1)	30.3(25.4)	1.6(1.6)	0.7	17.9 (0.7)	4.9	38.8	66.8
July	34	796	3184	99.5	0.3	99.7	63.6(14.6)	34.2(29.4)	3.8(1.0)	0.3	7.5	0.8	32.4	45.0
July	38	860	3440	65.6	34.6	66.0	61.2(11.4)	38.6(27.9)	1.4	1.6	5.3	11.6	28.6	39.3
				Most Posterior Chambers (MPC)										
November	20	502	502		0.8	1.2	19.1(14.3)	6.4(4.8)	6.4(0.8)	3.2	9.2	13.5	40.2	19.9

Table 3. - continued

December	26	678	678			0.8	14.5	30.7(12.4)	9.1(2.9)	1.8	13.3	7.4	15.3	15.3
January	44	1102	1102				0.9(0.2)	9.3(5.4)	10.5(2.0)	7.6	15.6	12.9	43.2	7.6
February	40	992	992			1.0	10.1(0.2)	11.5(0.4)	14.1(0.2)	6.0	12.1	15.3	29.8	0.8
March	42	940	940				0.2(0.2)	8.5(3.6)	12.6	8.9	14.3	22.8	32.8	3.8
April	32	738	738			3.8(1.4)	5.4(0.8)	13.0(4.6)	8.4(0.3)	8.7	10.8	20.1	29.8	7.1
May	34	798	798			0.5	6.0(0.8)	17.8(7.8)	4.5	4.8	15.5	18.8	32.1	8.6
June	26	614	614				17.6(9.8)	19.5(16.3)	1.3(1.3)	0.7	17.3 (0.7)	4.9	38.8	28.1
July	34	796	796			0.5	32.2(6.5)	23.1(19.1)	3.5(0.8)	0.3	7.5	0.5	32.4	26.1
July	38	860	860			1.9	24.2(6.3)	25.6(19.5)	1.4	1.2	5.3	11.2	28.6	25.8
				2nd Most Posterior Chambers (2nd MPC)										
November	20	502	502	2.0	3.6	37.1	51.0(21.5)	1.6(0.8)	2.0(0.4)	0.4	1.2	1.2		22.7
December	26	678	678	6.8	10.6	38.6	23.3	14.7(2.9)	1.8	0.9	2.1	1.2		2.9
January	44	1102	1102			9.1	40.5(1.6)	38.5(19.8)	7.1(2.0)	2.0	0.9	2.0		23.4
February	40	992	992	1.0	4.6	19.4	30.6(0.4)	27.6(2.0)	10.1(0.4)	2.0	1.8	2.8		2.8
March	42	940	940			11.7	29.1(0.9)	38.1(14.7)	11.9(0.4)	5.1	2.5	1.5		15.0
April	32	738	738	4.1	0.0	22.5	30.6(2.4)	34.7(12.7)	3.8(0.8)	2.2	1.6	0.5		15.9
May	34	798	798	0.3	0.3	25.6	37.1(5.5)	30.3(15.0)	1.8	1.5	2.3	1.0		20.5

Table 3. - continued

June	26	614	614		0.3	36.2	51.8(28.3)	10.7(9.1)	0.3(0.3)	0.0	0.7	0.0	37.7
July	34	796	796	0.5	0.0	55.8	32.2(8.3)	11.1(10.3)	0.3(0.3)	0.0	0.0	0.3	18.9
July	38	860	860	1.9	13.3	36.7	34.2(4.4)	13.0(8.4)	0.0	0.5	0.0	0.5	12.8

* Identification according to King's terminology (King et al., 1956)

** Number in parenthesis represents relative frequencies of degenerated egg chambers.

Table 4. **Profile of the developmental condition of the ovaries of D. mimica collected in Kipuka Ki during Nov. 1967-July 1968 and July 1972.**

Month of collection	Number of flies analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage* (relative frequencies in %)										% of degenerated egg chambers (Total)
				S5*	S6	S7	S8	S9	S10	S11	S12	S13	S14	
				Total Egg Chambers (TEC)										
November	48	1132	4528	61.3	38.7	89.8(0.2)**	43.8(10.2)	25.8(17.8)	9.0(3.4)	0.7	7.6(0.6)	11.0	30.2	32.2
December	20	554	2216	61.7	38.3	67.1	51.6(2.9)	47.7(22.4)	16.2(5.4)	2.5	14.8	5.1	41.2	30.7
January	66	1680	5920	95.6	5.6	96.1	64.6(1.4)	48.8(27.5)	19.0(3.7)	3.9(0.2)	17.3	8.0	27.7	32.9
February	20	526	2104	63.1	36.9	63.5(0.4)	54.8(2.3)	45.6(4.6)	19.0(0.8)	1.1	8.4(0.4)	8.4	15.6	8.4
March	50	1188	4752	73.6	26.4	74.1	48.5(4.4)	44.4(20.6)	26.1(1.0)	6.2(0.2)	14.8	26.8	45.1	25.9
April	46	1156	4624	96.2	3.8	96.2(1.9)	49.5(4.7)	43.4(16.1)	10.0(0.7)	15.7	7.6	19.4	49.5	23.4
May	44	1044	4176	99.8	0.6	99.0	47.1(2.3)	53.4(10.7)	2.7	10.3	19.2	20.9	27.2	13.0
June	24	580	2320	99.7	0.0	99.3	64.5(22.4)	27.6(17.6)	3.8(1.4)	3.8(0.3)	10.3	11.0	43.8	41.7
July	20	452	1808	98.2	2.2	98.2	52.2(6.2)	53.1(39.8)	9.7(4.4)	0.0	4.4(0.4)	0.9	68.6	50.9
July	84	1858	7432	75.3	23.7	75.9	72.4(18.6)	22.2(15.8)	0.5	2.5	9.9	10.5	31.4	34.4
				Most Posterior Chambers (MPC)										
November	48	1132	1132	0.7	1.1	7.4	13.9(4.4)	20.8(14.5)	7.4(2.3)	0.7	7.6(0.6)	10.9	30.2	21.7
December	20	554	554				7.6(0.4)	18.1(4.3)	13.0(4.0)	2.5	14.8	2.9	41.2	8.7

Table 4. - continued

January	66	1680	1680				6.1(0.4)	22.9(14.5)	15.6(2.7)	3.6(0.2)	17.0	7.7	27.1	17.9
February	20	526	526			0.4	16.0	33.8(2.3)	18.3(0.8)	1.1	6.5	8.4	15.6	3.1
March	50	1188	1188			0.2	0.3(0.3)	6.2(2.0)	12.5(0.3)	4.5(0.2)	10.8	20.4	45.1	2.9
April	46	1156	1156			0.5(0.3)	5.9(0.9)	9.3(2.6)	4.0(0.3)	9.3	6.6	14.9	49.5	4.2
May	44	1044	1044			0.6	3.4	23.0(3.1)	2.1	8.6	16.3(0.2)	18.8	27.2	3.3
June	24	580	580			2.1	14.8(5.0)	15.2(7.9)	3.4(1.0)	3.4(0.3)	9.3	7.2	44.5	15.2
July	20	452	452				5.3(0.4)	15.0(10.6)	6.6(3.5)	0.0	4.4(0.4)	0.0	68.6	15.0
July	84	1858	1858			1.8	31.0(8.2)	14.0(9.5)	0.5	2.4	9.1	7.9	33.1	15.7
			2nd Most Posterior Chambers (2nd MPC)											
November	48	1132	1132	4.2	6.0	54.5(0.2)	29.1(5.6)	4.4(3.2)	1.6(1.1)	0.0	0.2	0.0	0.0	10.1
December	20	554	554	0.0	3.6	23.1	40.4(2.5)	28.2(16.2)	2.9(1.4)	0.0	0.0	1.8	0.0	20.2
January	66	1680	1680	0.0	0.0	26.3	44.2(1.1)	25.5(13.1)	3.2(0.8)	0.4	0.2	0.2	0.0	15.0
February	20	526	526	0.0	4.9	44.1(0.4)	36.9(2.3)	11.8(2.3)	0.8	0.0	1.5(0.4)	0.0	0.0	5.3
March	50	1188	1188	0.2	0.0	11.6	29.3(2.7)	33.7(16.3)	13.3(0.7)	1.7	3.9	6.4	0.0	19.9
April	46	1156	1156	0.5	0.0	20.2	29.1(2.8)	32.4(11.9)	6.2(0.5)	6.2	1.0	4.3	0.0	16.3
May	44	1044	1044	0.6	0.0	28.4	35.8(0.6)	28.2(5.9)	0.6	1.5	3.4(0.4)	2.1	0.0	6.9
June	24	580	580	2.8	0.0	35.5	44.5(15.5)	12.8(9.3)	0.3(0.3)	0.3	0.3	3.4	0.0	25.2
July	20	452	452	0.0	0.0	20.4	39.4(5.3)	36.7(28.3)	2.7(0.4)	0.0	0.0	0.9	0.0	34.1
July	84	1858	1858	1.8	11.7	36.8	38.2(10.1)	7.7(6.1)	0.0	0.1	0.7	2.7	0.1	16.2

* Identification according to King's terminology (King et al., 1956).
**Numbers in parenthesis represents relative frequencies of degenerated egg chambers.

profile of ovarian development remains the same from month to month and from locality to locality, significant quantitative changes in the relative frequencies of some developmental stages and in the fraction of degenerated egg chambers were observed (Table 2-4). To evaluate the statistical significance of these differences I decided to combine the 14 stages into three functional groups based on morphological characteristics which also express the physiological performance of the egg chambers.

Group I includes the very young egg chambers (S4-S7) involved primarily in DNA synthesis required for the high polyploidization of the nurse cell nuclei (Painter and Reindorp, 1939). These are easily recognizable by their spherical shape, the uniformity of size between nurse cells and oocyte, and the absence of yolk from the oocyte. Group II combines all the stages involved primarily in RNA metabolism and yolk deposition (S8-S12). These are elongated chambers with well formed nurse cells, and a large oocyte containing vast amounts of yolk protein. Group III includes stages 13 and 14 which are egg chambers involved in the formation of the protective membranes (vitelline and chorion) and the mature eggs. In the following I shall refer to these groups as : DNA-stage; RNA-yolk stage; and chorion stage. To demonstrate that this classification does not alter the earlier conclusions, I have presented the developmental profiles for the December collection in Figure 3b. It can be seen that once again the chorion stage for the TEC was represented in approximately half of the ovarioles (56.8%), while both RNA-yolk and DNA stages were represented by 2 or 3 egg chambers per ovariole. The MPC were involved in either chorion synthesis (54.2%) or in RNA-yolk synthesis (45.8%), and the 2nd MPC were primarily involved in RNA-yolk (70.3%) and DNA synthesis (27.1%). The 3rd and 4th MPC (not shown in Figure 3b) were involved almost exclusively in

DNA synthesis. It is also clear that degeneration takes place exclusively during the process of RNA-yolk synthesis.

Analysis of the monthly collections according to the new classification for KPI (Appendix 1) demonstrates that the relative frequencies of the various developmental stages varied between collections, that these changes followed a defined pattern and were interrelated to each other. The clearest correlation in the TEC was observed between the RNA-yolk stage and the degenerated egg chambers, which were each changing in such a way that the increased frequency of one stage was accompanied by a decreased frequency of the other (Figure 4). These patterns of change were more pronounced when we compared the MPC with the 2nd MPC (Figure 5). Similar results were found in KPII (Appendix 2) and KK (Appendix 3). Summarizing these comparisons, it appears that accumulation of mature eggs in ovarioles caused 1) an increase of degenerated egg chambers in the 2nd MPC and a decrease of these in the MPC, 2) an increase of chambers synthesizing RNA-yolk in the 2nd MPC and a decrease of these in the MPC, and 3) a decrease of DNA stage in the 2nd MPC.

In order to understand the biological and ecological significance of these findings it was essential that we first determine the mode of reproduction for the species under controlled laboratory conditions.

3. Mode of reproduction of D. mimica under laboratory conditions

The growth rate of ovarian development, the profiles from flies of various ages, and the ovipositing behavior of wild collected flies were the parameters of reproductive mode analyzed.

a. Growth rates and profiles of ovarian development of young flies

Twenty females of different sizes collected in nature were kept individually in shell vials and their offspring were used to determine the

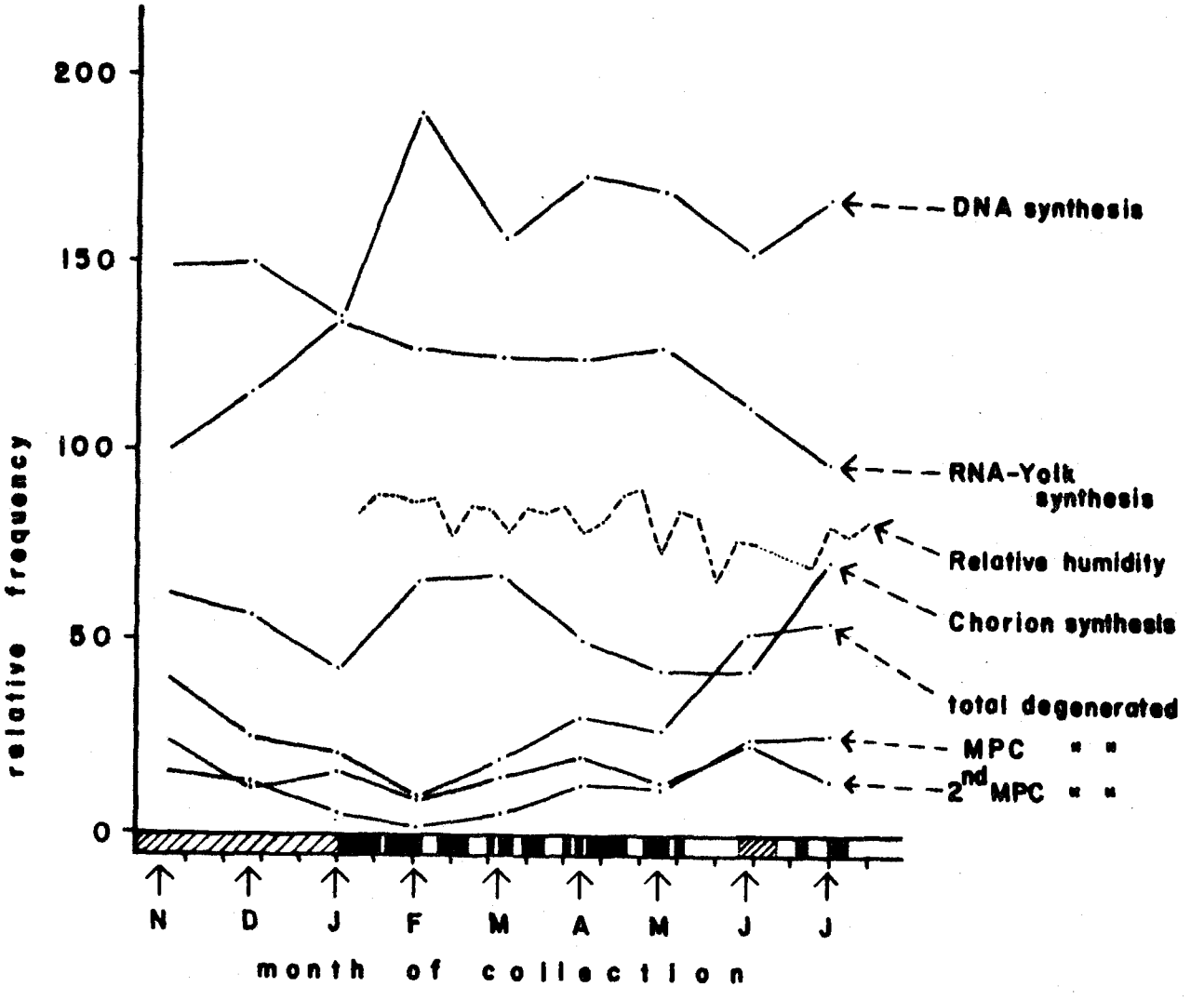

FIG. 4. Profile of ovarian development of D. mimica in Kipuka Puaulu I between November, 1967 and July, 1968 (total egg chambers). The mean relative humidity is also indicated. Constant high relative humidity (above 75-80%) is shown on the X-axis by solid blocks. Open blocks indicate low relative humidity and striated ones an absence of observations. (Only live egg chambers were included in the computation for the DNA, RNA-yolk and chorion stages; values above 100% indicate that more than one egg chamber in a given developmental stage was present in each ovariole, for details see text).

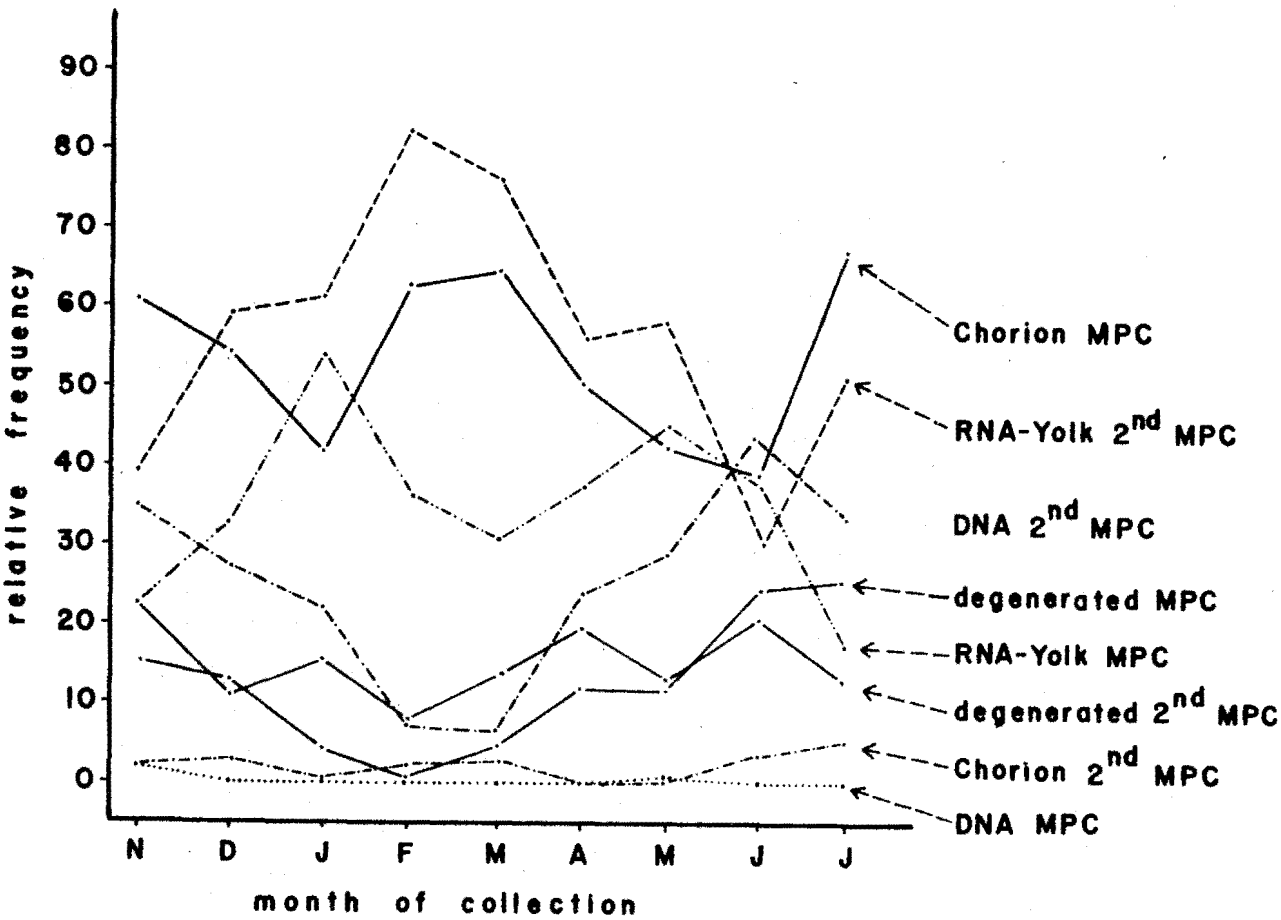

FIG. 5. Annual profile of ovarian development of D. mimica in Kipuka Puaulu I between November, 1967 - July, 1968. (Most posterior chambers and 2nd most posterior chambers). Only live egg chambers were included in computing the relative frequencies of DNA, RNA-yolk and chorion stages. (Values are given in %).

rate of ovarian growth. A minimum of 20 F1 females with a uniform adult age ($\pm$ 2 hours) were dissected at 24 hr. intervals for a period of 10 days, and their ovaries were analyzed. The remaining females were dissected after a period of 34 days to determine the profile of ovarian development in old flies. The parental flies were maintained on laboratory food for a period of 34 days to establish their oviposition behavior. At the termination of the experiment, their ovaries were analyzed to observe possible developmental disturbances caused by the laboratory environment.

The results demonstrated that newly eclosed flies do not contain egg chambers (Table 5). The first egg chamber was formed within 24 hours, the second one by the third day (Table 5, 2nd MPC) and thereafter a new one was added every second day until maturity was attained on the tenth day. The rate of ovarian growth increased almost linearly until the tenth day (Figure 6).

The growth rates of the 2nd MPC were similar to the MPC except for an initial delay in development for 3 days (Figure 6), a slight decrease of growth rate during the 8th-10th days and the presence of a few degenerated egg chambers in nine and ten day old flies which was reflected by the presence of mature eggs in the MPC.

The detailed profile of ovarian development from mature ten day old flies (Figure 7) was in excellent agreement with the profile of the natural population (Figure 3) indicating that our field observations were representing the analysis of the mature fraction of the mature fraction of the population.

b. Developmental profiles of old flies

The profile of the parental population after a month on laboratory food showed significant modifications (Figure 8). Mature eggs were present

Table 5. Profile of the developmental condition of the ovaries of 0-10 days old D. mimica.

Age of flies (days)	Number of flies analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage* (relative frequencies in %)												% of degenerated egg chambers (Total)
				S2*	S3	S4	S5	S6	S7	S8	S9	S11	S12	S13	S14	
				Total Egg Chambers (TEC)												
0(3 hrs)	28	?	?	no egg chamber												0.0
1	38	1064	1064	63.0	31.5	5.5										0.0
2	34	852	852	11.9	38.1	50.0										0.0
3	36	1004	1756		59.2	58.8	51.6	11.0	0.2							0.0
4	22	642	1284			74.5	24.0	36.1	64.2	1.9						0.0
5	24	698	1434			15.5	80.2	23.8	65.9	17.2	0.6					0.0
6	20	628	1654			6.6	89.0	10.6	90.0	32.9	36.2	2.0				0.0
7	18	560	1480				98.9	1.1	98.9	43.9	53.9(1.8)**	3.6	3.2	0.4		1.8
8	26	662	2514				97.9	1.6	98.4	53.0	55.1(2.4)	14.7	12.3	12.3	9.7	0.3	2.4
9	24	748	3002				98.7	1.3	98.7	48.9(2.1)	53.7(4.8)	9.9	4.8	10.7	35.8	28.9	6.9
10	30	894	3566				92.4	7.2	93.3	48.3(0.4)	58.2(10.1)	6.5	1.8	13.0	20.4	59.3	10.5
				Most Posterior Chambers (MPC)												
0(3 hrs)	28	?	?	no egg chambers												

Table 5. - continued

1	38	1064	1064	63.0	31.5	5.5									0.0
2	34	852	852	11.9	38.1	50.0									0.0
3	36	1004	1004		4.2	29.5	44.6	19.1	2.6						0.0
4	22	642	642					34.0	64.2	1.9					0.0
5	24	698	698				4.0	19.2	59.0	17.2	0.6				0.0
6	20	628	628					6.3	24.6	30.9	36.2	2.0			0.0
7	18	560	560						5.7	33.2	53.9(1.8)	3.6	3.2	0.4	1.8
8	26	662	662						0.5	5.8	41.3(2.4)	17.1	12.6	11.8	10.5	0.3	2.4
9	24	748	748							2.4(0.8)	12.0(2.1)	7.0	3.7	10.2	35.8	28.9	2.9
10	30	894	894						0.2	3.1	11.2(2.5)	2.2	0.9	9.2	13.9	59.3	2.5
				2nd Most Posterior Chambers (2nd MPC)													
0(3 hrs)	28	?	?	no egg chambers													
1	38	1064	1064	no 2nd MPC													
2	34	852	852	no 2nd MPC													
3	36	1004	752	10.4	50.0	34.5	5.2										0.0
4	22	642	642		11.2	63.3	23.4	2.2									0.0
5	24	698	698			19.2	69.3	4.6	6.9								0.0
6	20	628	628			6.3	23.9	3.6	64.1	2.0							0.0
7	18	560	560			5.7	0.0	83.2	83.2	11.1							0.0

Table 5. - continued

8	26	662	662	0.5	0.0	43.7	45.0	10.8						0.0
9	24	748	748			15.2	38.8(1.1)	41.7(2.9)	2.9	1.1	0.3			4.0
10	30	894	894	0.2	0.7	18.1	24.4(0.4)	41.4(7.6)	4.0	0.9	3.8	6.5		8.0

* Identification according to King's terminology (King et al., 1956).

** Numbers in parentheses represent relative frequencies at degenerated egg chambers.

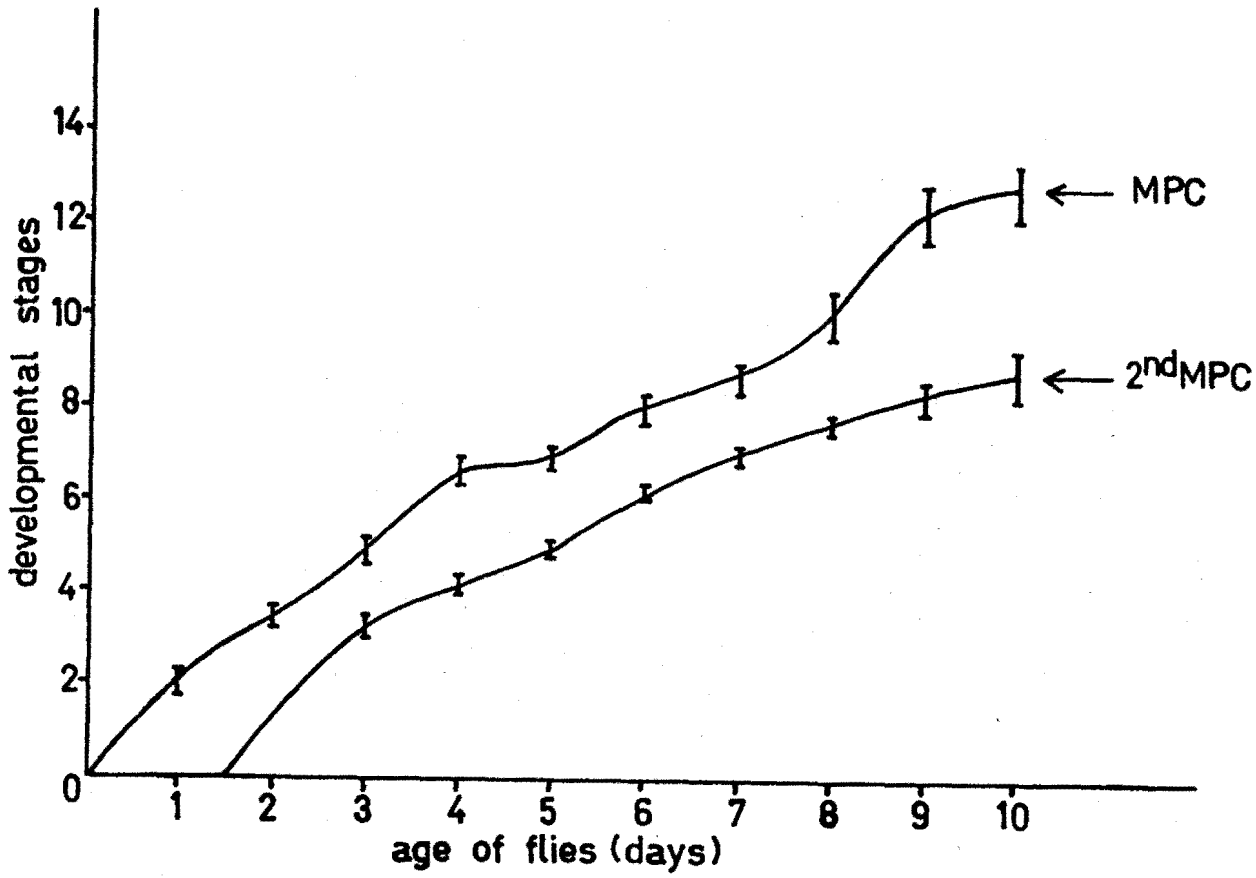

FIG. 6. Growth rates of ovarian development of D. mimica. Bars indicate the range.

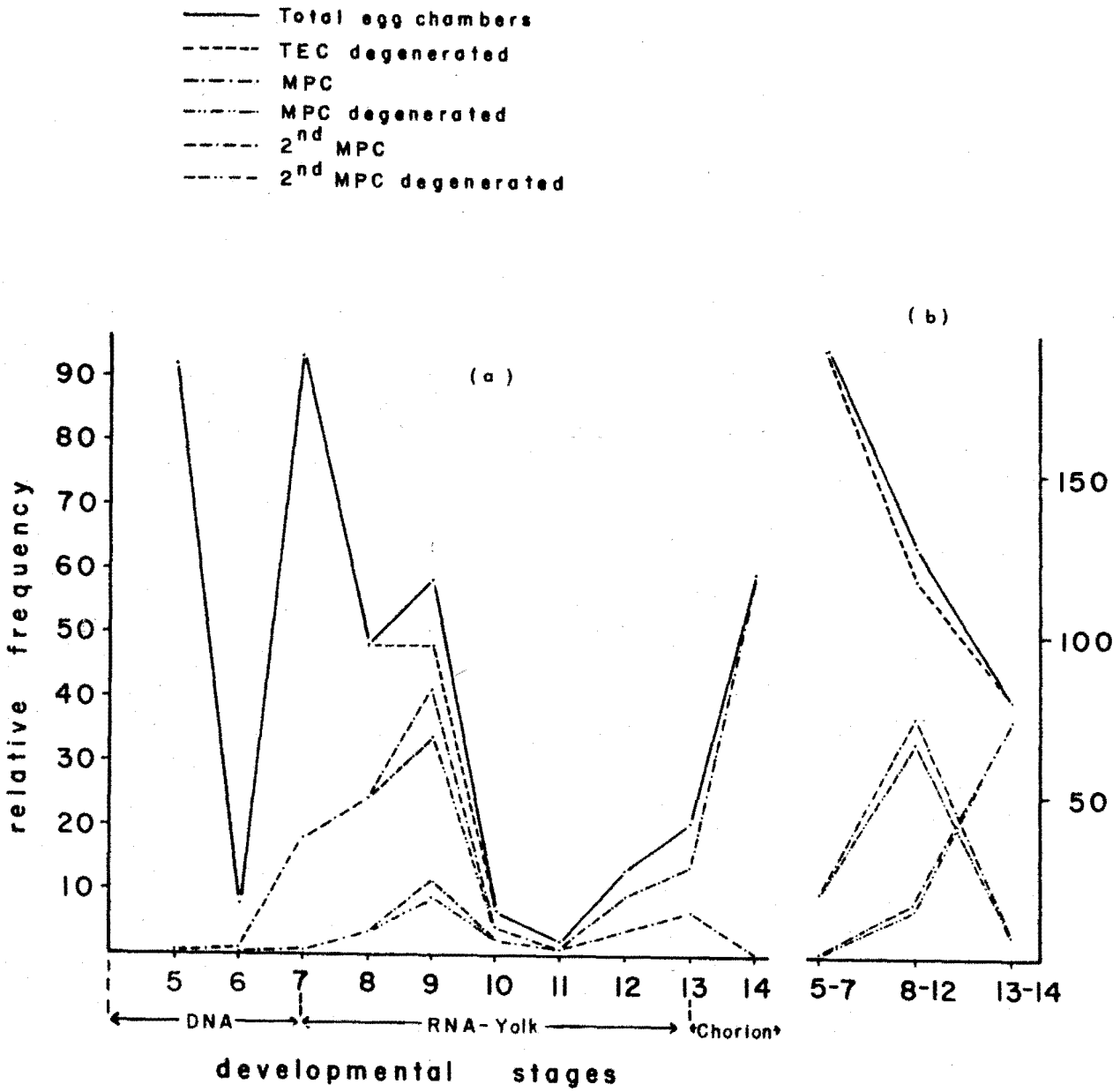

FIG. 7. Profile of ovarian development of 10 days old F_1 *D. mimica*. a) analytical profile based on 14 stages. b) summary profile based on 3 developmental groups.

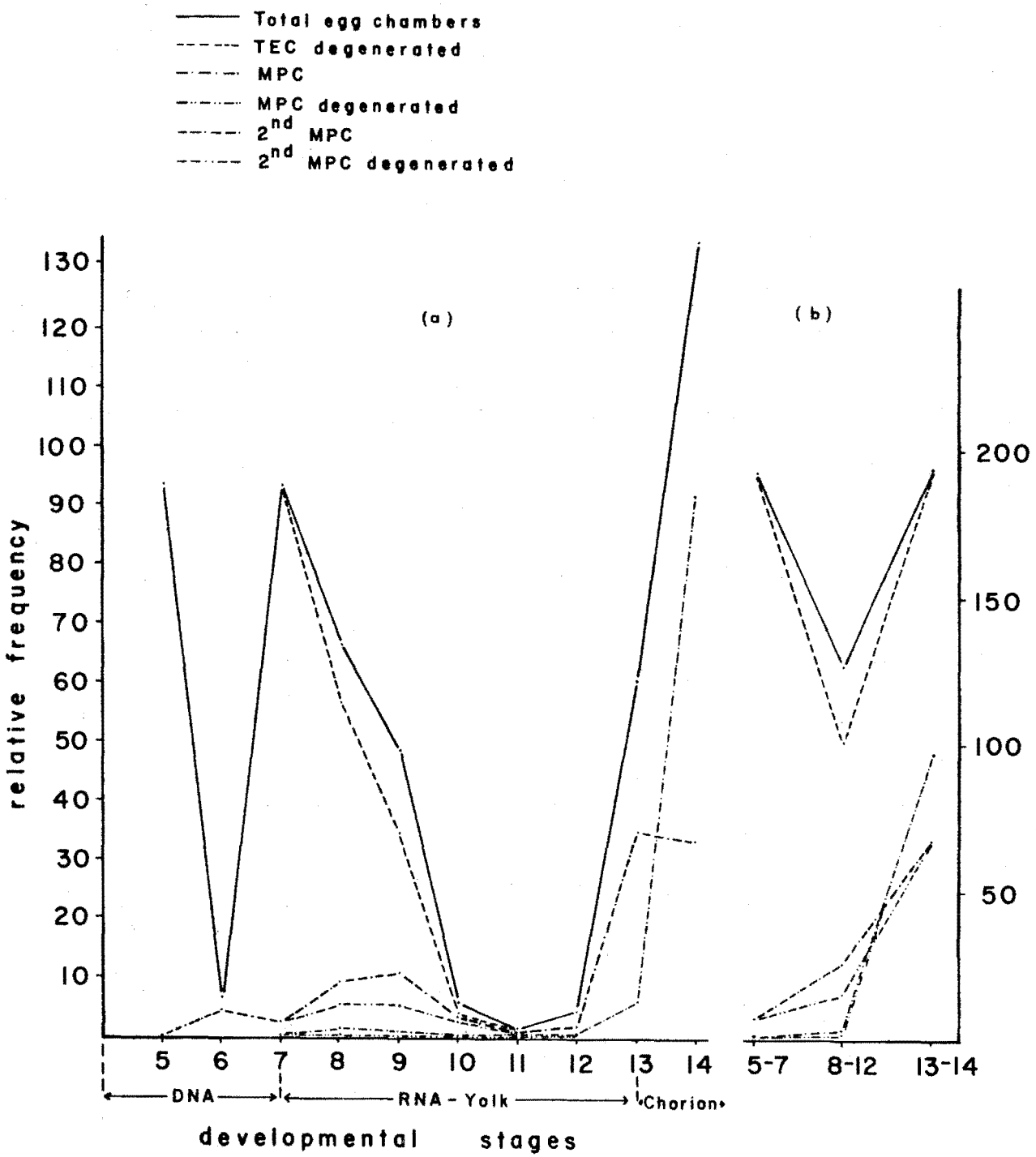

FIG. 8. Profile of ovarian development of old D. mimica after one month on laboratory feed. Notice the increase frequency of S13-14 stages in comparison to FIG. 2 and 9.
a) analytical profile. b) summary profile.

in almost all the ovarioles (98%) and in several of them (35%) two mature eggs were found (Figure 8). Virtually no MPC and very few 2nd MPC were degenerated. There were only 25% of 2nd MPC at RNA-yolk stage, 6.8% at DNA-stage and 68% at the chorion stage, compared to the 70%, 25%, and 5% observed both in young flies and in natural populations. The MPC were almost exclusively at the chorion stage (98%).

The profile of the old females of the F_1 generation also showed an accumulation of mature eggs in each ovariole (Figure 9). However, quite frequently these eggs were abnormal and had very little chorion, if any. The frequency of abnormal eggs was increased among F_1 individuals with numerous ovarioles (Figure 10a) which were also the larger individuals, and was independent of the size and ovariole number of the parental flies (Figure 10b). It should be pointed out that only a fraction of the F_1 flies produced abnormal eggs and among these flies some had only a few abnormal eggs, although in others virtually every egg was abnormal. These observations seem to indicate that under extremely favorable nutritional conditions, presumably the RNA and yolk are synthesized at a higher rate than the chorion, causing an imbalance in the normal oocyte maturation, resulting in chorion-deficient eggs. On very rare occasions we have observed such a situation in nature, and it is conceivable that it serves as a device to prevent overpopulation when extremely favorable nutritional environments pertain in nature.

c. Pattern of oviposition

The ovipositing behavior of 41 females of various sizes collected from natural populations was determined for a period of 34 days. The eggs of each female were collected every 24 hours and the mean number of eggs per fly per day was computed for the entire population (Figure 11). It was

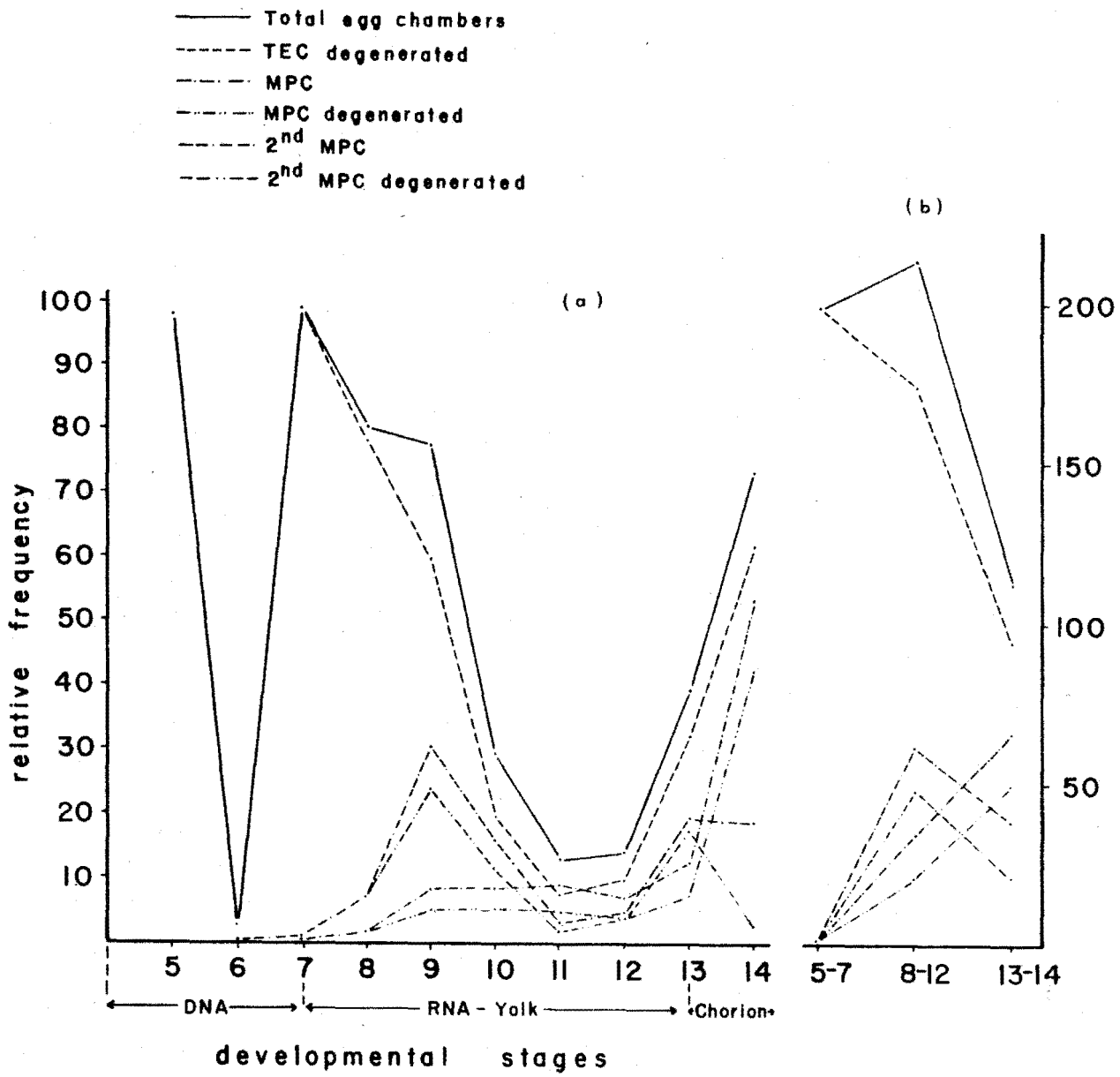

FIG. 9. Profile of ovarian development of old F_1 female D. mimica. Notice the presence of degenerated S13-14 representing abnormalities of chorion formation. a) analytical. b) summary profiles.

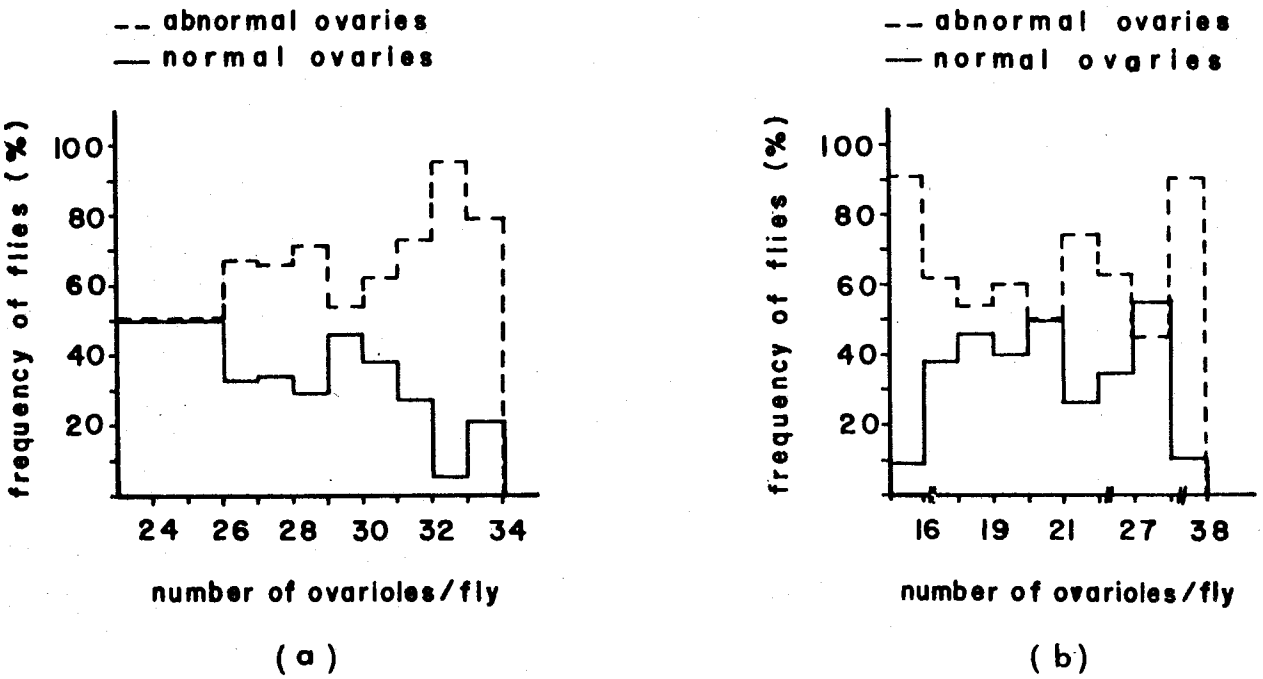

FIG. 10. Distribution of ovaries with chorion deficient eggs in laboratory strains D. mimica. a) correlation between number of ovarioles and ovarian abnormality for each fly. b) correlation between number of ovarioles of the parental flies and ovarian abnormality in the F_1 generation.

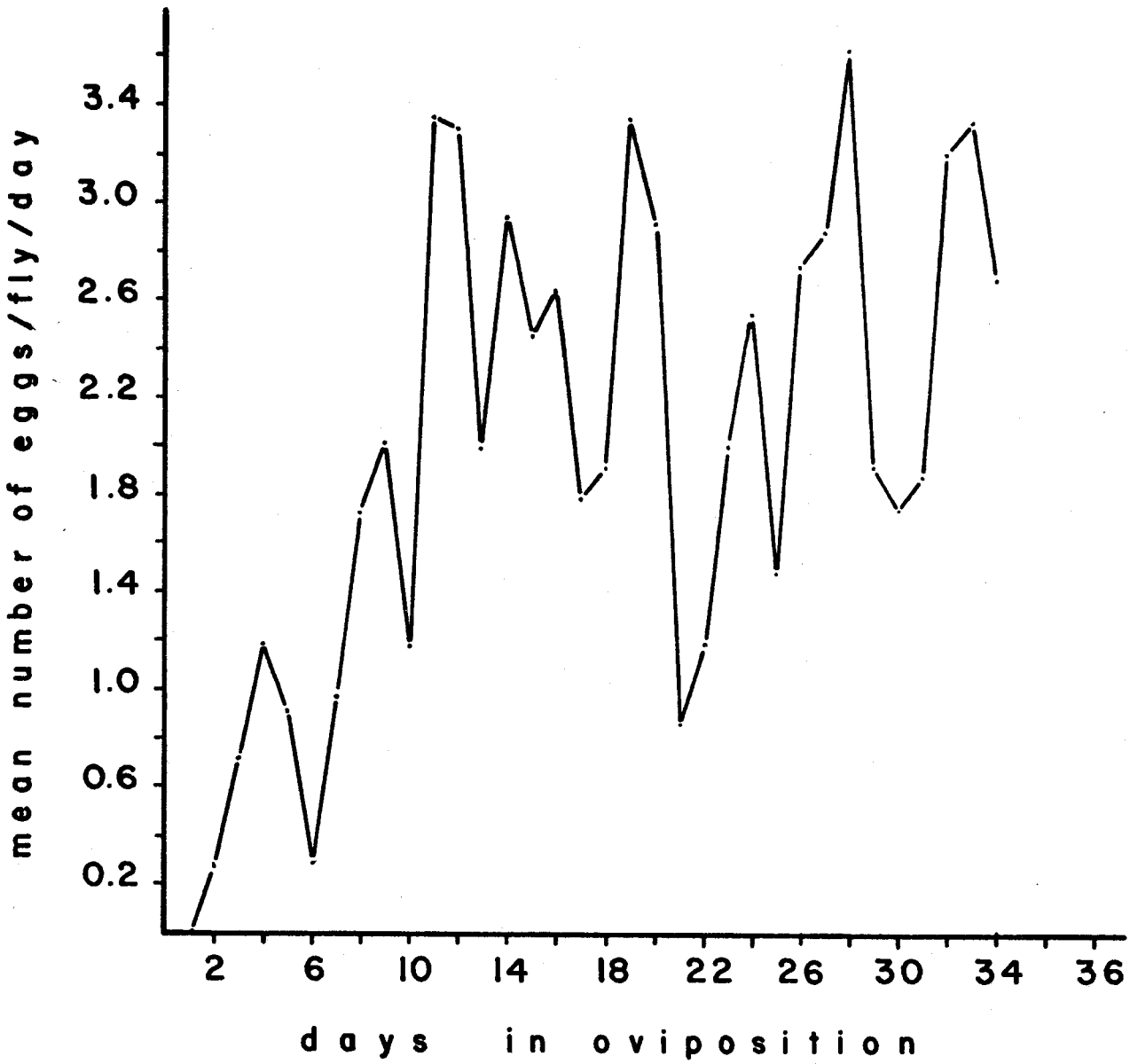

FIG. 11. Pattern of oviposition in D. mimica.

Table 6. Pattern of oviposition in D. mimica

Number of female line	Total number of eggs laid	Duration of oviposition (days)	Mean number of eggs per day	Maximum number of eggs laid in 1day	Maximum number of eggs laid in sequence	Size of thorax (mm)
1 (P2)	0	1	0	0	0	1.7
2 (P18)	0	2	0	0	0	1.8
3 (P19)	0	3	0	0	0	1.8
4 (P16)	0	3	0	0	0	1.6
5 (P9)	0	4	0	0	0	1.6
6 (P17)	0	4	0	0	0	1.7
7 (P27)	0	4	0	0	0	1.6
8 (P5)	0	13	0	0	0	1.3
9 (PK6)	3	21	0.14	2	2 (1)*	1.6
10 (PK9)	7	33	0.21	4	4 (1)	1.7
11 (P1)	8	33	0.24	3	3 (1)	1.7
12 (PK14)	10	29	0.34	4	4 (1)	1.9
13 (PK12)	12	16	0.75	5	6 (2)	1.8
14 (P6)	15	33	0.45	5	6 (2)	1.4
15 (P13)	23	33	0.70	7	8 (2)	1.3
16 (P3)	30	33	0.91	6	8 (3)	1.8
17 (PK2)	33	33	1.00	5	12 (3)	1.5
18 (P4)	34	33	1.03	7	7 (1)	1.3
19 (P26)	34	33	1.03	9	11 (2)	2.0
20 (P25)	40	33	1.21	8	14 (2)	2.0
21 (P7)	44	26	1.69	6	34 (11)	1.4
22 (P10)	46	33	1.39	11	14 (2)	1.6
23 (PK13)	50	33	1.51	7	22 (7)	1.8
24 (PK11)	53	23	2.30	8	18 (5)	1.8
25 (P20)	57	33	1.73	10	20 (3)	1.9
26 (P8)	59	33	1.79	8	15 (4)	1.4
27 (PK1)	60	33	1.82	8	22 (5)	1.3
28 (P23)	61	23	2.09	10	31 (6)	1.9
29 (PK4)	62	33	1.88	12	12 (1)	1.5
30 (P14)	66	33	2.00	10	12 (2)	1.4
31 (P22)	69	33	2.09	11	32 (9)	1.9
32 (PK8)	78	33	2.36	11	31 (6)	1.6
33 (P15)	86	33	2.61	16	33 (5)	1.5
34 (PK15)	87	33	2.64	11	35 (6)	1.9
35 (P21)	102	19	5.37	14	80 (10)	1.9
36 (P11)	107	33	3.24	33	33 (1)	1.7
37 (PK7)	113	21	5.38	21	67 (8)	1.6
38 (PK5)	120	33	3.64	16	20 (2)	1.5
39 (P24)	135	33	4.09	26	56 (6)	2.0
40 (PK10)	146	33	4.42	14	40 (6)	1.8
41 (P12)	226	33	6.85	24	66 (6)	1.7

* Numbers in parenthesis represent the successive days of oviposition for each sequence.

consistently observed that for an initial period of ten days, fecundity was very low (mean number of eggs per fly per day = 0.76 versus 2.37 for the other 24 days). This was attributed to the adaptation of the flies to the new environment, and also to the fact that a fraction of the initial population consisted of immature flies which require 8-10 days for ovarian maturation (Figure 6). The ovipositing activity of the population for the entire period of observation was unexpectedly low, representing only a fraction (1/4) of the fecundity potential of this species. This was partially reflected by the great variability observed among individual flies in their ovipositing behavior. It was observed that individual flies tended to oviposit several eggs on successive days, followed by a rest prior to new oviposition. Considering this ovipositing behavior, we estimated the maximum number of eggs laid by a fly in a day or over consecutive days (Table 6), and found that the majority of the flies had a realized fecundity comparable to their fecundity potential, (27 out of 41 flies laid 5-15 eggs in a day which is equal to the range of mature eggs per fly in a natural population). In fact, a small number of flies far exceeded their fecundity potential and laid more eggs than their mean number of ovarioles (mean number of ovarioles per fly = 23.9 with a range 16.0-29.0; maximum number of eggs for two females 30-35). We suspect that these individuals are of great importance in maintaining the reproductive plasticity of the population. The size of the female had no influence on its ovipositing behavior (Table 6, Column 7).

4. Microclimatic conditions in the collecting localities

The environmental parameters, atmospheric and soil temperature, relative humidity, rainfall, and potential evaporation, which would have

an effect on the development of the ovaries were recorded by Dr. Smathers (1968) and are summarized here.

a. Air temperature and relative humidity

Air temperature and relative humidity (RH) were recorded simultaneously for periods of seven days. The percentage of time during the week when temperature (Table 7) or RH (Table 8) was within specified ranges was estimated and the weekly mean values were computed. The maximum and minimum values were also recorded. A graphic presentation of the weekly mean values is shown in Figure 12. Unlike other tropical forests characterized by a constant high RH and temperature, we have observed in our collecting sites a slight progressive increase in mean temperature from 15°C in January to 18°C in July and a parallel decrease in RH from 88% in January to 78% in July. In addition to these gradual changes, abrupt fluctuations of RH and/or temperature were often caused by rainfall and these fluctuations were of the utmost importance for the reproductive physiology of the flies.

b. Gradients of air, soil and surface temperature

Temperature of soil, surface of ground and Sapindus fruits and leaves, which are known habitats at various stages of the life cycle of D. mimica (Heed, 1968; Kambysellis, 1974), were recorded at various occasions and correlations were made with the activity and reproductive ability of flies. The results summarized in Table 9 indicated that under the forest canopy there was very little or no difference in temperature from ground level to 250 cm above. The soil temperatures by contrast, were usually lower than the atmospheric ones and exhibited a significant vertical-gradient decrease of 5-8°C from ground level to 5 cm below. These differences were less

Table 7. Weekly temperature in Kipuka Puauli I - between January 12-July 29, 1968. The values given are percent of time during the week that temperature was in the given percent range.

Range Temperature (C^o)	1/12-15	1/15-22	1/22-29	1/29-2/5	2/5-12	2/12-19	2/19-26
7.2-10.0	10.5	0.0	0.0	2.3	1.2	4.9	0.0
10.1-12.8	36.8	8.4	16.7	24.4	14.1	30.5	8.6
12.9-15.6	26.3	49.4	60.7	61.6	63.5	40.2	42.0
15.1-18.4	26.3	42.2	22.6	11.6	21.2	19.5	48.1
18.5-21.2	0.0	0.0	0.0	0.0	0.0	4.9	1.2
21.3-24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mean	13.6	15.3	14.6	13.9	14.5	14.3	15.6
range	9.4-18.3	11.1-18.3	10.5-18.3	10.0-16.7	9.4-17.2	9.4-19.4	12.2-18.9

Range Temperature (C^o)	2/26-3/4	3/4-11	3/11-18	3/18-25	3/25-4/1	4/1-8	4/8-15
7.2-10.0	2.3	2.4	0.0	0.0	0.0	0.0	2.5
10.1-12.8	6.0	17.6	0.0	14.5	11.9	14.1	14.8
10.9-15.6	27.4	36.5	71.8	57.8	50.0	36.5	56.8
15.7-18.4	63.1	29.4	24.7	22.9	38.1	40.0	23.5
18.5-21.2	1.2	14.1	3.5	4.8	0.0	9.4	2.5
21.3-24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mean	16.0	15.6	15.5	15.1	15.2	15.7	14.8
range	10.0-18.9	10.0-21.1	13.3-19.4	11.7-18.9	10.5-18.3	11.1-20.0	9.4-20.0

Range Temperature (C^o)	4/15/22	4/22-29	4/29-5/6	5/6-13	5/13-20	5/20-27	5/27-6/3
7.2-10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.1-12.8	3.5	0.0	18.2	5.9	5.9	2.4	1.2
12.9-15.6	45.9	68.7	45.4	39.3	22.4	35.7	40.0
15.7-18.4	50.6	31.3	31.8	44.0	49.4	26.2	32.9
18.5-21.2	0.0	0.0	4.5	10.7	22.4	35.7	25.9
21.3-24.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
mean	15.8	15.5	14.9	16.2	16.8	17.2	16.7
range	11.7-18.3	13.3-17.8	11.1-18.9	11.7-18.9	10.5-20.0	12.7-21.1	12.7-20.6

Range Temperature (C^o)	6/3-10	6/10-24	6/24-7/1	7/1-8	7/8-15	7/15-22	7/22-29
7.2-10.0	0.0		0.0	0.0	0.0	0.0	0.0
10.1-12.8	0.0		1.2	2.4	0.0	0.0	0.0
12.9-15.6	22.9	unit	39.3	20.5	25.6	12.9	12.5
15.7-18.4	42.2	out of	28.6	54.2	40.7	57.6	50.0
18.5-21.2	30.1	order	27.4	22.9	32.6	29.4	33.8
21.3-24.0	4.8		3.6	0.0	1.2	0.0	3.8
mean	17.7		17.0	17.2	17.6	17.6	18.2
range	13.9-22.8		12.7-22.2	12.7-20.6	13.3-21.7	15.0-21.1	15.0-22.2

Table 8. - Weekly relative humidity in Kipuka Puaulu I - between January 12-July 29, 1968. The values given are percent of time during the week that RH was in the given percent range.

Range Humidity %	1/12-15	1/15-22	1/22-29	1/29-2/5	2/5-12	2/12-19	2/19-26
90-100	34	64	69	34	36	1	16
80- 89	41	31	23	58	56	56	77
70- 79	12	2	8	7	6	26	4
60- 69	12	1			1	11	4
50- 59		1				4	
40- 49						2	
30- 39							
range	58-99	56-93	70-93	68-93	62-92	42-93	64-92
mean	83.8	88.4	88.3	86.9	87.2	77.6	85.7

Range Humidity %	2/26-3/4	3/4-11	3/11-18	3/18-25	3/25-4/1	4/1-8	4/8-15
90-100	18	4	19	31	12	7	25
80- 89	70	60	62	46	71	66	52
70- 79	10	18	12	17	16	11	18
60- 69	2	14	5	2	1	13	2
50- 59		5	1	4		2	2
40- 49							
30- 39							
range	60-91	50-91	55-91	50-93	60-91	47-91	56-93
mean	84.8	78.6	84.9	83.7	85.4	78.7	81.2

Range Humidity %	4/15-22	4/22-29	4/29-5/6	5/6-13	5/13-20	5/20-27	5/27-6/3
90-100	62	79	1	51	28	0	5
80- 89	34	21	42	36	55	31	43
70- 79	2		34	8	14	24	25
60- 69	1		18	5	1	15	19
50- 59			5		1	17	8
40- 49						10	
30- 39							
range	65-92	80-92	52-91	60-92	54-93	40-88	48-93
mean	88.2	90.0	73.5	84.7	82.7	65.9	76.2

Range Humidity %	6/3-10	6/10-24	6/24-7/6	7/6-8	7/8-15	7/15-22	7/22-29
90-100	5		0	13	17	6	2
80-89	50		32	55	37	61	56
70-79	21	Unit	32	14	25	25	25
60-69	10	out of	13	14	11	8	13
50-59	9	order	21	5	10		4
40-49	1		2				
30-39	4						
range	30-94		44-90	50-91	48-92	58-92	50-93
mean	75.9		69.7	80.2	77.8	81.1	78.1

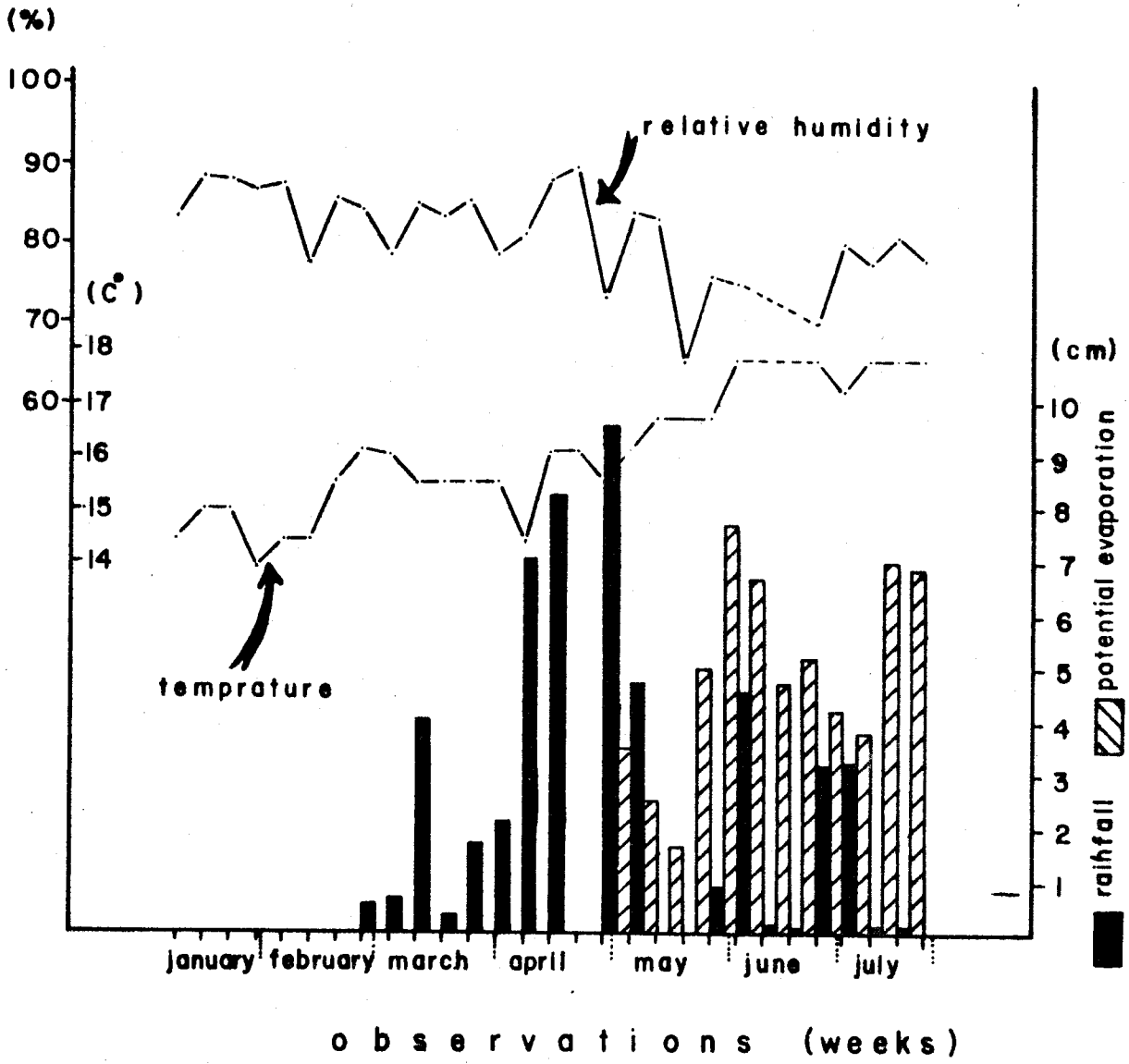

FIG. 12. Graphic presentation of relative humidity (RH) and temperature during March-July 1968 in Kipuka Puaulu.

Table 9.- Gradient of Air, Soil, and Surface temperature (C^{O}) and Relative Humidity (RH) of Kipukas for various periods. Measurements were taken at the same location in each Kipuka near midday.

Distance above (+) or below (-) ground	Date and Location 2/15/68 Kipuka Puaulu I Temp.	RH	Kipuka Puaulu II Temp.	RH	Kipuka Ki Temp.	RH	3/6/68 Kipuka Puaulu I Temp.	RH	Kipuka Puaulu II Temp.	RH	Kipuka Ki Temp.	RH	3/16/68* Kipuka Puaulu I Temp.	RH	Kipuka Puaulu II Temp.	RH	Kipuka Ki Temp.	RH
Air & Soil:																		
+250cms.	18.1	42	21.1	36	22.0	40	18.6	60	16.6	72	18.1	58	13.0	93	13.6	86	14.0	94
+150cms.	18.8	45	21.0	43	21.1	40	19.0	60	17.0	76	19.6	60	13.2	93	13.3	80	14.0	94
+50cms.	18.3	47	21.0	45	21.5	44	19.6	64	17.0	82	19.8	60	13.4	94	14.0	90	14.5	96
ground level	17.8	60	21.0	47	21.1	45	19.0	75	17.6	88	20.3	64	14.0	94	14.5	88	14.5	94
-0.5cms.	14.1		17.1		17.3		15.0		14.8		14.0		14.0		15.0		15.0	
-2.5cms.	13.3		14.0		15.5		14.1		13.1		14.0		15.2		15.3		15.0	
-5.0cms.	12.3		13.1		14.0		14.8		13.6		15.3		15.2		15.3		15.5	
Surface of:																		
Tree trunk +150cms.	16.0		20.0		19.1		18.1		14.5		17.3		13.0		13.5		14.0	
Under leaf +150cms.	17.8		21.6		21.6		18.0		16.5		17.5		13.0		14.0		14.0	
Tree trunk + 50cms	16.1		20.0		18.0		18.8		15.6		16.5		14.0		13.5		14.0	
Under leaf + 50cms.	17.0		22.1		21.6		18.0		16.1		18.6		13.8		13.8		14.0	
Humus	14.8		18.0		17.1		16.1		14.5		17.6		14.0		15.0		15.0	
Brown leaves	15.3		2-.3		20.3		17.6		15.1		17.5		14.4		14.0		15.0	
Brown Sapindus fruit	15.3		20.0		20.5		17.0		15.0		17.1		14.2		14.0		14.8	
Green Sapindus fruit	15.5		18.0		20.0		17.0		15.0		17.0							

Table 9. - continued

Distance above (+) or below (-) ground	Date and Location: 4/1/68 Kipuka Puaulu I Temp.	RH	4/1/68 Kipuka Puaulu II Temp.	RH	4/1/68 Kipuka Ki Temp.	RH	5/23/68 Kipuka Puaulu I Temp.	RH	5/23/68 Kipuka Puaulu II Temp.	RH	5/23/68 Kipuka Ki Temp.	RH
Air & Soil:												
+250cms.	17.8	77	16.9	78	17.5	76	21.3	49	22.8	46	23.3	53
+150cms	17.8	76	17.0	78	18.0	75	21.6	50	22.5	51	23.5	52
+ 50cms.	18.0	76	17.2	79	19.0	78	23.1	57	22.1	58	24.0	52
ground level	18.2	76	18.5	80	18.9	80	22.1	61	22.5	61	23.5	56
-0.5cms.	16.2		15.5		17.0		18.1		18.5		18.0	
-2.5cms.	15.2		15.0		16.0		16.7		16.5		16.3	
-5.0cms	15.0		14.9		15.5		15.0		15.1		15.3	
Surface of:												
Tree trunk +150 cms.	18.0		16.7		17.0		20.3		21.8		22.0	
Under leaf +150cms.	17.5		16.8		16.9		20.3		21.5		21.5	
Tree trunk + 50cms.	17.7		16.7		17.0		21.0		21.5		20.0	
Under leaf + 50cms.	17.5		16.7		17.1		20.0		20.0		21.0	
Humus	17.5		16.5		17.0		19.6		19.5		20.5	
Brown leaves	17.7		16.7		16.9		19.8		19.1		20.5	
Brown Sapindus fruit			16.7				20.0		19.0		20.5	
Green Sapindus fruit					17.0							

* All measurements were taken immediately after a rainfall and under a cloud cover.

Table 10 - Weekly records of rainfall in Kipuka Puaulu I and Kipuka Ki between March 3 - July 29, 1968. Values are given in inches.

Date	Kipuka Puaulu I under canopy (3960')*	Kipuka Puaulu I outside canopy (3960')*	Kipuka Ki outside canopy (4250')*
March 3-11	0.19	0.32	0.56
18	0.26	0.74	0.90
25	1.60	1.72	1.78
Total March	2.05	2.78	3.24
April 1	0.13	1.25	1.54
8	0.60	1.06	1.02
15	0.80	0.90	1.00
23	2.76	4.75	5.25
29	3.25	6.00	5.75
Total April	7.54	13.96	14.56
May 7	0.00	0.06	0.06
13	3.76	5.74	5.75
20	1.80	1.75	2.20
29	0.00	0.00	0.00
Total May	5.56	7.55	8.01
June 3	0.00	0.05	0.04
10	0.34	0.54	0.60
17	1.79	2.20	2.25
24	0.05	0.16	0.20
Total June	2.18	2.95	3.09
July 2	0.04	0.05	0.05
8	1.24	1.75	1.75
15	1.26	2.20	2.40
22	0.05	0.15	0.20
29	0.05	0.04	0.08
Total July	2.64	4.19	4.48
Grand Total (March-July)	19.97	31.43	33.38

*Elevation above sea level

pronounced in the stations KPII and KKI, which as we indicated, have a less dense forest, particularly during the periods of defoliation of Sapindus (January-March).

The RH exhibited a clear vertical-gradient increase of 5-18% from 250 cm above to ground level, and RH was higher in the collecting site KPI with the more dense vegetation.

By comparison to the kipukas, the microclimatic conditions a few feet away outside of the forest canopy were dramatically different. For example, the air temperature 2 cm above ground level was 16°C higher than that at 150 cm, and soil temperatures were decreasing very slowly, and only at depths of 8-10 cm were equal to the air temperature at 150 cm. This observation clearly indicates that the dense vegetation in the kipukas stabilizes the microenvironments, which in turn allows the flies to stabilize their populations and oviposit almost undisturbed for the entire year.

c. Rainfall

Weekly-measurements of rainfall in the collecting localities were started the second week of March, 1968. Comparable accumulations of rainfall were recorded in all three collecting sites (Table 10) with a consistent, slightly higher accumulation in Kipuka Ki. The weekly measurements showed significant seasonal variability, with April-May being the wettest period within the time recorded. Of the rain falling on the forest canopy in Kipuka Puaulu, approximately 65% reaches the forest floor. This "through-fall" rain is very important for the Drosophila populations, and varies considerably between observations, determined by factors such as

seasonal canopy density, fog interception by the canopy, wind-driven rain intercepted by the vegetation, and cloud cover affecting evaporation rate.

d. pH at Rainwater

The acid fumes of the erupted volcano increased the acidity of the rainwater and acidity was more pronounced when the trade winds blew the acid fumes toward the kipukas. Thus from January to mid-April, 75% of the tests revealed pH ranges between 3.5-5.0, and 25% of the tests were in the normal range of 5.0-7.7, while from mid-April through June only 20% of the tests showed pH 3.5-5.0. Recordings of pH 7.0 or higher were obtained when trade winds prevailed, and readings of 7.7-7.9 when the trade winds were very strong. We were not able to detect an effect of the low pH on ovarian development of D. mimica. However, isozyme studies in these populations have indicated a possible effect on the allele frequencies at an acid phosphatase locus (Rockwood, 1969).

e. Potential evaporation

Moisture loss from the vegetation and forest floor varied considerably during the recording period (Figure 12). The highest potential evaporation was found as expected in Kipuka Ki and primarily during the period when very little or no rain fell. It should be pointed out the although Kipuka Ki received more rain than Kipuka Puaulu (Table 10), by having a higher potential evaporation (maximum of 7.9 cc/day), its relative humidity was equalized or slightly decreased. This evapotranspiration in the kipukas is very low if compared to an open area, as for example Kilauea Iki, an area of higher rainfall 2 miles east of Kipuka Ki, where values as high as 36 cc/day have been reported (Smathers, personal communications).

DISCUSSION

The reproductive state of D. mimica populations in their natural habitats remained constant. The profile of ovarian development, determined by the relative frequencies of the developmental condition of each egg chamber in the ovary, was found to be basically the same over a nine months' test period and also in an additional sampling after four years. The mean number of ovarioles per fly, a species characteristic value, remained constant (23.8) with a slight variation ($\pm$4.21) which was reflected by variation in the size of the flies (mean thorax size 1.8 $\pm$ 0.18 mm) attributed primarily to larval competition (Kambysellis and Heed, 1971). Each ovariole possessed four to seven egg chambers at various stages of egg development. The fourteen developmental stages characterizing the egg maturation (King et al., 1956) were represented only in a fraction of the ovarioles and were never found more than once in the same ovariole. This was valid not only for the eggs in early developmental stages but also for the mature eggs. Very infrequently mature eggs could be found in all the ovarioles of an individual female, but never in all the ovarioles of the population (Table 2-4). Specific developmental stages (S5, S7, S9) were usually more frequent than others (S6, S8, S10, S13), which suggested the existence of a mechanism regulating the development of the oocytes in accordance with the pre-existing developmental stages. The hierarchical localization of the egg chambers on the ovariole according to their developmental condition, i.e., the most posterior chambers (MPC) were the most advanced stages, 2nd MPC younger stages, 3rd MPC still younger, etc. (Table 2-4), and the asynchronous development between ovarioles, further reinforced the existence of a control mechanism operating via communication between developing oocytes, ovarioles and within the organism as a whole.

It is suspected that such communications are of a hormonal and/or neural nature, the predominant channels of cell communication in insects (Williams, 1969).

The ability of an individual to control the rate of egg development and/or the number of eggs which reach maturity, by interrupting the entire developmental process when disadvantageous environmental conditions occur, is of the utmost importance for the fitness of the populations. This is because a great deal of energy required for the completion of egg maturation can be conserved by interrupting the developmental process.

We have found the D. mimica possess the ability to regulate the number of egg chambers reaching maturity by interrupting the developmental process at stages 8-9, by causing degeneration of the nurse cell nuclei, and by resorbance of the egg chambers (Table 2-4). To fully understand the efficiency of such a regulatory mechanism, we need to remember that the major objects of the organism during oogenesis are: 1) the accumulation of sufficient nutrients (yolk) into the egg to be utilized as a source of energy and as precursors for macromolecular synthesis during the entire period of embryogenesis (Davidson, 1968), 2) the accumulation of sufficient genetic instructions (probably as mRNA and rRNA) for protein synthesis during the initial period of embryogenesis (Gross, 1967), accomplished by selective DNA replication and transcription of the genome (Davidson, 1968), and 3) the formation of protective memberanes (chorion, vitelline membrane), to insure protection and survival of the egg-embryo from the vicissitudes of its environment.

The syntheses of these important macromolecules are spaced throughout oogenesis, with each one assigned almost exclusively to a given developmental stage (Figure 13). Since the entire process apparently functions in a chain-like reaction, the interruption of the synthesis of any one of the

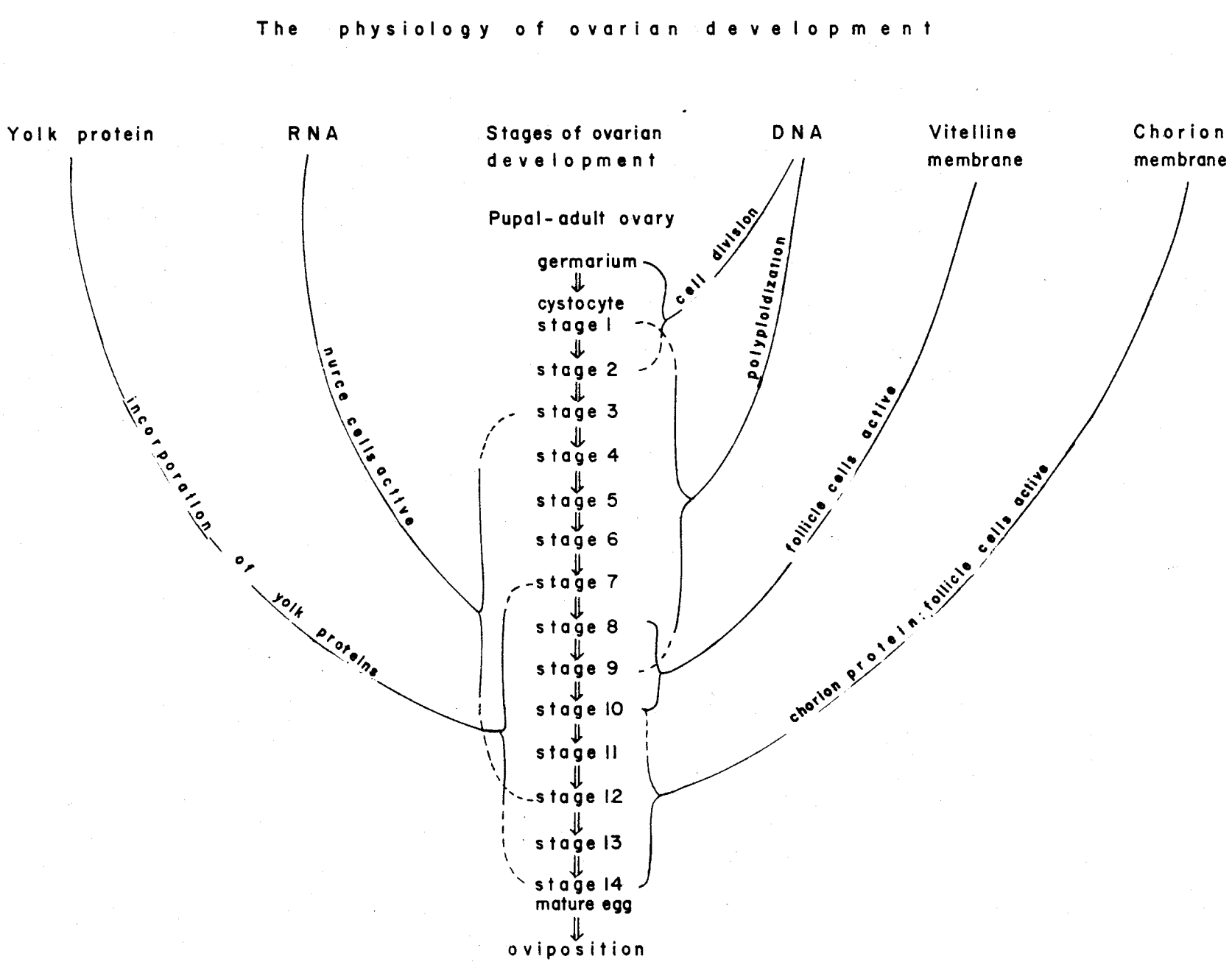

FIG. 13. Schematic presentation of ovarian development of Drosophila indicating the major physiological events during oogenesis.

macromolecules would prevent the synthesis of subsequent molecules and, therefore, would interfere with the reproductive efficiency of the individual. By estimating the energy invested for the synthesis of each macromolecule, and their quantities synthesized during oogenesis, it is suspected that the most energy consuming molecules are in sequence, yolk proteins, RNA, membrane proteins and DNA. Our field observations with D. mimica demonstrated that interruption of oogenesis probably takes place during late stages of DNA synthesis and/or sometime early in RNA-yolk protein synthesis (cf Figure 13). This clearly indicates that the individuals are capable of interrupting their oocyte development at an early stage when very little energy has already been invested.

The necessary signals for initiating and terminating these biosynthetic processes were suspected to be triggered by environmental stimuli. The accurate daily records of the environmental parameters with some potential in regulating the ovarian development (i.e., temperature, relative humidity, rainfall, and potential evaporation) revealed that relative humidity was the most important factor. Constant high relative humidity (above 75-80%) for a certain period of time was essential for constant and normal egg maturation (Figure 4). Low relative humidity could be tolerated by the flies, but only for a short period of 2-4 days. Prolonged dry conditions for more than a week resulted in a high degree of oocyte degeneration, a dramatic decrease of RNA-yolk synthesis and a moderate decrease of DNA synthesis (Figure 4, June-July). Our observations suggested that the tolerance of flies to changing environments or alternatively, the efficiency of flies in registering environmental changes required a period of approximately one week. That is to say if the relative humidity changes from high to low and remains low for a week, the flies would respond by preventing the maturation of new oocytes. If, on the other hand, the RH

changes from low to high and remains high for a week, the flies would reinitiate development of new oocytes. It is of interest to point out that this seven day lag of environmental tracking corresponds to the time required for the young oocytes to reach the stage of RNA-yolk synthesis (Figure 6). Therefore, the oocytes degenerating at the end of tolerant periods have been exposed to the modified RH during their entire life. Whether during this period they have undergone severe modification in their physiological performance (primarily in the selective DNA replication) is not known at the present.

The target organs at which the RH acts are unknown. It is, however, conceivable and it is suspected that the RH acts on the endocrine system of the fly by somehow regulating the secretion of juvenile hormone which is known to control the uptake of yolk protein in several insects (for review see Wyatt, 1972) including Drosophila (Kambysellis and Heed, 1974).

We have observed that RH also has an influence on the frequency of egg chambers at the stage of chorion synthesis. However, this effect could be masked if the flies possessed the ability to retain some mature eggs for a period of a few days and oviposited them even when a short period of rainfall or high humidity became available. With such an assumption it is expected that the frequencies of degenerated egg chambers would fluctuate between MPC and 2nd MPC following the fluctuation of RH and rainfall. Therefore, by observing the developmental condition of the ovaries of a population in their natural habitats we could determine, with a degree of accuracy, the pre-existing environmental conditions. We have used our field developmental data to make predictions of the existing environmental conditions before and during each collection for one of our collecting localities (Figure 4). Thus according to the previous assump-

tions, we anticipate that a) if we have a population with numerous eggs, a high frequency of degenerated 2nd MPC, and a low frequency of degenerated MPC, (as, for example, the collection of March 4, Figure 4), then the weather must have been consistently dry for the last week prior to collection, i.e., the last week of February (our prediction was confirmed, see Figure 4, Figure 12,); b) if there were few mature eggs with numerous degenerated MPC and few 2nd MPC (Figure 4, April collection), then the weather must have been consistently dry during the week prior collection, followed by a recent wet or humid period (Table 10, observation of last week of March; Figure 12); c) if the 2nd MPC were also degenerated (Figure 4, June collection), then the dry weather conditions must have been prolonged for more than two weeks (last two weeks of May, Figure 12); d) if the degeneration of MPC and 2nd MPC is high, accompanied by an accumulation of mature eggs (Figure 4, July), then the atmosphere must have remained dry for at least two or three weeks (May-June, Figure 4, Figure 12); and e) if the number of mature eggs is high, yet the degeneration rather low (Figure 4, February), then the weather must have been consistently humid for the last two weeks prior to collection (January, Figure 4, Figure 12). On the basis of these observations it is clear that although the mode of reproduction for D. mimica remained in general the same from population to population and from year to year, some of its aspects were temporarily modified by environmental parameters to compensate for environmental stresses without severe threat to the existence of the population (Kambysellis, 1974). This implies that long term evolutionary forces have operated and established a genetic basis for the reproductive mode for the species, allowing at the same time a degree of reproductive plasicity and

the tolerance to environmental conditions in D. mimica was strickingly different from those found in other species which are known to occupy different ecological habitats (Kambysellis, 1974; Kambysellis and Heed, 1971).

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Appendix I. Profiles of the developmental condition of the ovaries of D. mimica collected in Kipuka Puaulu I during Nov. 1967-July 1968 and July-August 1972

Month of collection	Number of files analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage (relative frequences in %) DNA (S4-S7)*	RNA-yolk (S8-S12)*	Chorion (S13+S14)*	% of degenerated egg chambers
				Total Egg Chambers (TEC)			
November	42	1090	4360	185.7	103.7	62.8	40.0
December	28	620	2480	176.8	129.1	56.8	24.5
January	26	628	2512	157.3	150.6	42.4	20.4
February	34	780	3120	198.0	163.3	65.4	9.2
March	28	570	2280	162.8	150.5	67.4	18.9
April	22	456	1824	197.4	132.0	50.4	30.3
May	28	580	2320	199.0	134.1	42.4	26.9
June	14	340	1460	198.2	115.3	42.4	52.4
July	14	342	1368	197.6	103.6	72.5	55.6
July	52	1186	4744	171.8	111.9	47.2	42.5
August	67	1623	6492	205.2	127.7	53.8	43.4
				Most Posterior Egg Chambers (MPC)			
November	42	1090	1090	2.0	37.1	60.9	15.2
December	28	620	620	0.0	45.8	54.2	12.9
January	26	628	628	0.0	58.3	41.7	4.4
February	34	780	780	0.0	37.2	62.8	0.5
March	28	570	570	0.0	35.4	64.6	4.6
April	22	456	456	0.0	49.6	50.4	12.2
May	28	580	580	0.7	56.9	42.4	11.8
June	14	340	340	0.0	61.2	38.8	24.1

Appendix I-continued

July	14	342	342	0.0	32.7	67.3	25.2
July	52	1186	1186	1.0	51.5	47.6	24.1
August	57	1623	1623	1.1	45.8	53.0	15.4
			2nd Most Posterior Chambers (2nd MPC)				
November	42	1090	1090	34.9	62.0	1.8	23.3
December	28	620	620	27.1	70.3	2.6	10.9
January	26	628	628	22.3	77.3	0.3	15.6
February	34	780	780	7.2	90.5	2.3	8.2
March	28	570	570	6.7	90.5	2.8	14.0
April	22	456	456	24.1	75.9	0.0	19.7
May	28	580	580	29.0	71.0	0.0	12.8
June	14	340	340	45.3	51.2	3.5	23.0
July	14	342	342	29.8	64.9	5.3	13.4
July	52	1186	1186	46.5	50.7	1.5	16.4
August	67	1623	1623	21.4	78.0	0.0	28.3

* Identification according to King's terminology (King et al. 1956)

Appendix II. Profiles of the developmental condition of the ovaries of D. mimica collected in Kipuka Puaulu II during Nov. 1967-July 1968 and July 1972

Month of collection	Number of files analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage (relative frequences in %) DNA (S4-S7)*	RNA-yolk (S8-S12)*	Chorion (S13-S14)*	% of degenerated egg chambers
			Total Egg Chambers (TEC)				
November	20	502	2008	184.1	102.4	55.0	42.7
December	26	678	2712	152.9	118.5	23.0	18.9
January	44	1102	4408	199.8	156.5	58.3	31.6
February	40	992	3968	172.4	144.7	48.0	4.0
March	42	940	3760	167.0	151.9	57.0	20.4
April	32	738	2972	196.2	127.1	50.7	23.3
May	34	798	3192	195.2	129.6	52.1	29.8
June	26	614	2456	198.3	120.9	43.6	66.8
July	34	796	3184	199.4	109.3	33.2	45.0
July	38	860	3440	166.2	108.1	40.2	39.3
			Most Posterior Egg Chambers (MPC)				
November	20	502	502	2.0	44.2	53.8	19.9
December	26	678	678	8.0	69.3	22.7	15.3
January	44	1102	1102	0.0	43.9	56.1	7.6
February	40	992	992	1.0	53.8	45.2	0.8
March	42	940	940	0.0	44.5	55.5	3.8
April	32	738	738	3.8	46.3	49.9	7.1
May	34	798	798	0.5	48.6	50.9	8.6
June	26	614	614	0.0	56.4	43.6	28.1
July	34	796	796	0.5	66.6	32.9	26.1
July	40	860	860	1.9	57.7	39.8	25.8

Appendix II-continued

			2nd Most Posterior Chambers (2nd MPC)				
November	20	502	502	42.7	56.1	1.2	22.7
December	26	678	678	56.0	42.8	1.2	2.9
January	44	1102	1102	9.1	88.9	2.0	23.4
February	40	992	992	25.0	72.1	2.8	2.8
March	42	940	940	11.7	86.8	1.5	15.0
April	32	738	738	22.5	72.9	0.5	15.9
May	34	798	798	26.1	72.9	1.0	20.5
June	26	614	614	36.5	63.5	0.0	37.7
July	34	796	796	56.3	43.4	0.3	18.9
July	40	860	860	51.9	47.7	0.5	12.8

* Identification according to King's terminology (King et al. 1956)

Appendix III. Profiles of the developmental condition of the ovaries of D. mimica collected in Kipuka Ki during Nov. 1967-July 1968 and July 1972

Month of collection	Number of files analyzed	Number of ovarioles analyzed	Number of egg chambers analyzed	Distribution of egg chambers according to their developmental stage (relative frequences in %) DNA (S4-S7)	* RNA-yolk (S8-S12)	* Chorion (S13-S14)	*	% of degenerat egg chambers
				Total Egg Chambers (TEC)				
November	48	1132	4528	189.8	86.9	41.2		32.2
December	20	554	2216	167.1	133.9	46.2		30.7
January	66	1680	5920	197.7	153.7	35.7		32.9
February	20	526	2104	163.5	128.9	24.0		8.4
March	50	1188	4752	174.1	140.0	71.9		25.9
April	46	1156	4624	196.2	126.3	68.9		23.4
May	44	1044	4176	199.4	132.8	48.1		13.0
June	24	580	2320	199.0	110.0	54.8		41.7
July	20	452	1808	198.6	119.5	69.5		50.9
July	84	1858	7432	174.9	107.5	41.9		34.4
				Most Posterior Egg Chambers (MPC)				
November	48	1132	1132	9.2	50.4	41.4		21.7
December	20	554	554	0.0	56.0	44.0		8.7
January	66	1680	1680	0.0	65.1	34.9		17.9
February	20	526	526	0.4	75.6	24.0		3.1
March	50	1188	1188	0.2	34.3	65.5		2.9
April	46	1156	1156	0.5	35.1	64.4		4.2
May	44	1044	1044	0.6	53.4	46.0		3.3

Appendix III-continued

June	24	580	580	2.1	46.2	51.7	15.2
July	20	452	452	0.0	31.4	68.6	15.0
July	84	1858	1858	1.8	57.0	41.0	15.7
			2nd Most Posterior Chambers (2nd MPC)				
November	48	1132	1132	64.7	35.3	0.0	10.1
December	20	554	554	26.7	71.5	1.8	20.2
January	66	1680	1680	26.3	73.5	0.2	15.0
February	20	526	526	49.0	51.0	0.0	5.3
March	50	1188	1188	11.8	71.8	6.4	19.9
April	46	1156	1156	20.7	75.0	4.3	16.3
May	44	1044	1044	29.0	69.5	2.1	6.9
June	24	580	580	38.3	58.3	3.4	25.2
July	20	452	452	20.4	68.7	0.9	34.1
July	84	1858	1858	50.3	46.7	2.8	16.2

* Identification according to King's Terminology (King et al. 1956)

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